Biocatalytic asymmetric synthesis of unnatural amino acids using C-N lyases

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CHAPTER 7

Biocatalytic Asymmetric Synthesis of *N*-Aryl-functionalized Amino Acids and Substituted Pyrazolidinones

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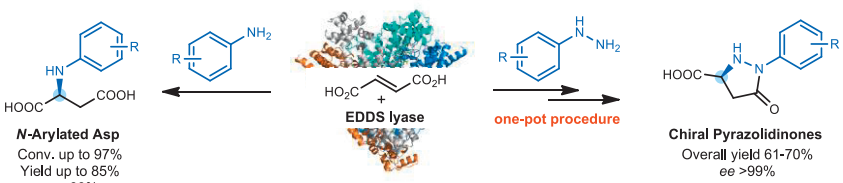

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Abstract

N-arylated α-amino acids and pyrazolidin-3-ones are widely being used as chiral building blocks for pharmaceuticals and agrochemicals. Here we report a biocatalytic route for the asymmetric synthesis of various *N*-arylated aspartic acids applying ethylenediamine-*N*,*N*'-disuccinic acid lyase (EDDS lyase) as biocatalyst. This enzyme shows a remarkably broad substrate scope, enabling the addition of a variety of arylamines to fumarate with high conversions, yielding the corresponding *N*-arylated aspartic acids in good isolated yields and with excellent enantiomeric excess (*ee* >99%). Furthermore, we developed a chemoenzymatic method towards synthetically challenging chiral 2-aryl-5-carboxyl pyrazolidin-3-ones, using arylhydrazines as bisnucleophilic donors in the EDDS lyase-catalyzed hydroamination of fumarate followed by an acid-catalyzed intramolecular amidation, achieving good overall yields and high optical purity (*ee* >99%). In addition, we successfully combined the EDDS lyase-catalyzed hydroamination and acid-catalyzed cyclization steps in one pot, thus providing a simple chemoenzymatic cascade route for synthesis of enantiomerically pure pyrazolidin-3-ones. Hence, these newly developed biocatalytic methods provide convenient alternative routes to important chiral *N*-arylated aspartic acids and difficult 2-aryl-5-carboxylpyrazolidin-3-ones.

Keywords

Asymmetric synthesis, biocatalysis, EDDS lyase, unnatural amino acids, pyrazolidinones.

Introductions

Optically pure functionalized α-amino acids are highly valuable as tools for biological research and as chiral building blocks for pharmaceuticals, nutraceuticals, and agrochemicals.[1–3] In particular, *N*-arylated α-amino acids are part of the core structures of a number of medicinally important agents, such as fibrinogen receptor antagonist Lotrafiban (**1**, Figure 1a)[4] and protein kinase C activator Indolactam-V (**2**, Figure 1a).[5,6] Despite their broad applications, the direct synthesis of chiral *N*-arylated α-amino acids remains a challenge. Current chemical strategies for the synthesis of enantioenriched *N*-arylated α-amino acids and their esters are mainly based on extending the existing free amino group of the Cα-stereocentre through Cu-catalyzed Ullmann-type coupling reactions[6–9], Pd-catalyzed *N*-arylation[10–13], and hypervalent iodine chemistry (Figure 1a).[14] However, they are limited by their poor atom economy, the use of heavy metals, and harsh reaction conditions that may result in partial or complete racemization of the α-stereocentre. Biocatalysis provides a valuable alternative route to chiral unnatural amino acids.[15–18] Previously reported enzymatic asymmetric synthesis of *N*-alkyl-functionalized α-amino acids were primarily based on two types of carbon-nitrogen bond-forming reactions: (i) conjugate addition of amines to the double bond of α,β-unsaturated acids catalyzed by various types of carbon-nitrogen lyases, including aspartate ammonia lyases (DALs)[19,20], methylaspartate ammonia lyases (MALs)[21,22], and the recently reported ethylenediamine-*N*,*N*'-disuccinic acid lyase (EDDS lyase)[23,24]; and (ii) reductive amination of α-keto acids with amines catalyzed by a number of oxidoreductases, such as reductive aminase (RedAm)[25], opine dehydrogenases (OpDHs)[26,27], *N*-methyl-amino acid dehydrogenases (NMAADHs)[28,29], ketimine reductases (KIREDs)[29,30], and Δ^1-pyrroline-5-carboxylate reductases (P5CRs)[29,31]. However, to the best of our knowledge, no enzymatic route has been reported for the synthesis of *N*-aryl-functionalized α-amino acids. Thus, the development of an efficient and sustainable biocatalytic methodology to enantiomerically pure *N*-arylated α-amino acid derivatives would be particularly desirable.

Pyrazolidin-3-ones and related five-membered dinitrogen-fused heterocycles are widely found as core framework in dyes, agrochemicals, and pharmaceutically active molecules, such as the very first synthetic analgesic and antipyretic drug Phenazone (**3**, Figure 1b)[32], lipoxygenase inhibitor Phendione (**4**, Figure 1b)[33], and anti-Alzheimer agents (**5**, Figure 1b).[34] In addition, chiral pyrazolidine-3-ones can also function as efficient catalysts in promoting Diels-Alder reactions[35], and catalyzing kinetic resolution of secondary alcohols and axially chiral biaryl compounds.[36] Due to their broad application in drug development, as well as in synthetic methodologies, several chemical methods have been developed for the synthesis of enantiomerically pure pyrazolidinones and related heterocycles,

including chemical[35,37] or kinetic resolution[38], 1,3-dipolar cycloaddition[39–41], Lewis acid catalyzed conjugate addition[42], and Pd-catalyzed asymmetric hydrogenation.[43] However, creating a biocatalytic methodology as alternative route to chiral pyrazolidinones is an as yet unmet challenge.

The enzyme ethylenediamine-*N*,*N*'-disuccinic acid lyase (EDDS lyase), from *Chelativorans sp.* BNC1, naturally catalyzes a reversible two-step sequential addition of ethylenediamine to two molecules of fumarate providing (*S*, *S*)-EDDS as final product.[44] We recently demonstrated that EDDS lyase could accept a wide variety of amino acids with terminal amino groups for regio- and stereoselective addition to fumarate, providing the natural product aspergillomarasmine A and various related aminocarboxylic acids.[23] In addition, EDDS lyase could also accept a number of (hetero)cycloalkyl-substituted amines, allowing the asymmetric synthesis of (*S*)-*N*-cycloalkyl-substituted aspartic acids.[24] Therefore, the remarkably broad nucleophile scope of EDDS lyase prompted us to further explore the less nucleophilic arylamines as novel non-natural substrates in the asymmetric hydroamination of fumarate, which would enable the production of chiral *N*-arylated aspartic acids as the corresponding enzymatic products. Moreover, we envisioned that chiral pyrazolidine-3-ones could be constructed by using arylhydrazines as bisnucleophilic donors in the EDDS lyase-catalyzed regio- and stereoselective addition to fumarate followed by a simple intramolecular amidation.

Herein we report a biocatalytic methodology for the synthesis of optically pure (*S*)-*N*-arylated aspartic acids in high conversions and isolated yields. Moreover, an efficient one-pot two-step chemoenzymatic route towards chiral pyrazolidine-3-ones has been developed. These strategies highlight a highly regio- and stereoselective hydroamination step catalyzed by EDDS lyase, offering alternative synthetic choices to prepare chiral *N*-arylated α-amino acids as well as chiral pyrazolidine-3-ones.

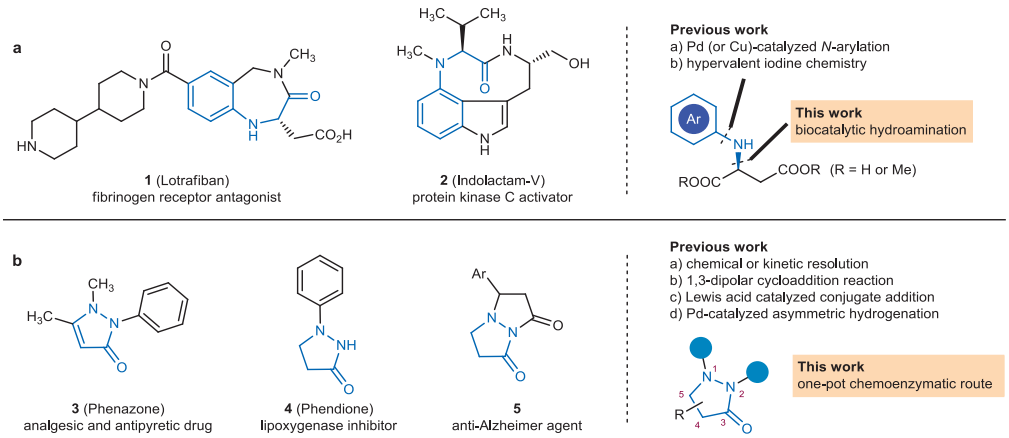


Figure 1. Biologically active compounds and synthetic strategies for the *N*-arylated amino acid (**a**) and pyrazolidine-3-one (**b**) precursors.

Results

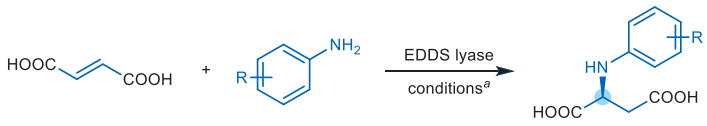
Biocatalytic synthesis of *N*-arylated aspartic acids

In contrast to aliphatic amines, aromatic amines are challenging substrates for biocatalytic addition reactions due to their relatively low nucleophilicity. Our previous study demonstrated that EDDS lyase could accept glycine as an unnatural substrate, facilitating the low nucleophilic α-amino group of glycine to function as the nucleophile in the amination of fumarate (**6**).[23] This prompted us to start our investigation by testing aniline (**7a**, Table 1, entry 1) as potential substrate in the EDDS lyase-catalyzed biotransformation. Remarkably, aniline (**7a**) was efficiently converted by purified EDDS lyase to afford *N*-phenyl-substituted aspartic acid (**8a**), with high conversion (91%) and good isolated yield (80%) using only 0.05 mol% biocatalyst loading under optimized conditions (Table 1). To determine the stereochemistry of enzymatic product **8a**, HPLC analysis on a chiral stationary phase was conducted by using chemically prepared authentic standards with known (*R*/*S*)- and (*S*)-configuration (Figure S2). This analysis revealed that product **8a** was present as a single (*S*)-configured enantiomer with excellent enantiomeric excess (*ee* >99%, Table 1, entry 1).

Next, the substrate scope was investigated by examining a panel of electronically diverse substituted anilines and heteroarylamines as unnatural substrates in the EDDS lyase-catalyzed amination of fumarate, as monitored by ^{1}H NMR spectroscopy (Table S1). We were pleased to find that EDDS lyase displayed a broad arylamine substrate scope which was,

as expected, affected by the electron-withdrawing/donating nature, position and bulkiness of the substituents on the aromatic ring (Table 1 and Table S1). Clearly, substitution of the aromatic ring at the *ortho*-position was not tolerated by the enzyme, for which only *o*-fluoroaniline (**7b**) gave a very low conversion (6%, Table 1 and Table S1). Impressively, arylamines with *meta*-substituents, including *m*-fluoroaniline (**7c**), *m*-toluidine (**7d**) and *m*-methoxyaniline (**7e**), were efficiently accepted by EDDS lyase providing the respective products **8c-e** (Table 1, entry 3-5). High conversions (87-97%), good isolated product yields (53-84%), and excellent stereoselectivity (*ee* >99%) were observed (Table 1, entry 3-5). Similarly, *para*-substituted arylamines, such as *p*-fluoroaniline (**7f**), *p*-toluidine (**7g**), *p*-methoxyaniline (**7h**), *p*-ethylaniline (**7i**), *m*,*p*-dimethylaniline (**7j**), and *p*-carboxylaniline (**7k**), were also well accepted by the enzyme, giving high to excellent conversions (75-95%) and yielding the corresponding amino acids **8f-k** (34-85% isolated yields) as the (*S*)-configured enantiomers with >99% *ee* (Table 1, entry 6-11). Noteworthy, *para*-halogenated anilines (**7l-n**) were also processed to deliver chiral (*S*)-*N*-haloarylaspartic acids (**8l-n**) with 82-96% conversions and 52-69% isolated yields (*ee* >99% in all cases, Table 1, entry 12-14), leaving the halogens available for potential downstream synthetic manipulation. The larger nucleophile *p*-isopropylaniline (**7o**) was a poor substrate for EDDS lyase, resulting in low conversion (17%, Table 1, entry 15). Arylamines bearing a strong electron-withdrawing group (such as *p*-nitro or *p*-CF_3) or electron-deficient heteroarylamines (such as pyridine-2-amine, pyridine-4-amine, and thiazol-2-amine) were not accommodated as substrates by EDDS lyase, most likely due to their diminished nucleophilicity (Table S1).

Table 1. Enzymatic synthesis of (*S*)-*N*-arylated aspartic acids.



6 **7a-o** **8a-o**

Entry	Arylamine	Product	R	Time [h]	Conv.[b] (yield[c]) [%]	*ee*[d] [%]
1	**7a**	**8a**	H	48	91 (80)	>99 (*S*)[f]
2	**7b**	**8b**	*o*-F	72	6 (n.d.[e])	–
3	**7c**	**8c**	*m*-F	48	91 (53)	>99 (*S*)[g]
4	**7d**	**8d**	*m*-Me	24	97 (84)	>99 (*S*)[g]
5	**7e**	**8e**	*m*-OMe	48	87 (53)	>99 (*S*)[g]
6	**7f**	**8f**	*p*-F	24	92 (85)	>99 (*S*)[f]
7	**7g**	**8g**	*p*-Me	48	75 (57)	>99 (*S*)[f]
8	**7h**	**8h**	*p*-OMe	48	92 (75)	>99 (*S*)[f]
9	**7i**	**8i**	*p*-Et	48	76 (53)	>99 (*S*)[g]
10	**7j**	**8j**	*m,p*-diMe	72	90 (70)	>99 (*S*)[g]
11	**7k**	**8k**	*p*-CO_2H	24	95 (34)	>99 (*S*)[g]
12	**7l**	**8l**	*p*-Cl	48	96 (63)	>99 (*S*)[g]
13	**7m**	**8m**	*p*-Br	48	95 (69)	>99 (*S*)[f]
14	**7n**	**8n**	*p*-I	48	82 (52)	>99 (*S*)[g]
15	**7o**	**8o**	*p*-iPr	72	17 (n.d.[e])	–

[a]Conditions and reagents: fumaric acid (**6**, 50 mM), arylamine substrates **7a-o** (10 mM) and purified EDDS lyase (0.05 mol% based on arylamine) in buffer (50 mM NaH_2PO_4/NaOH, pH 8.5), with 5% DMSO as cosolvent at room temperature. [b]Conversions were determined by comparing ^{1}H NMR signals of substrates and corresponding products. [c]Isolated yield after cation-exchange chromatography. [d]Enantiomeric excess (*ee*) was determined by HPLC on a chiral stationary phase using racemic standards. [e]Not determined owing to low conversion, the product formation was confirmed by comparison of ^{1}H NMR data of a crude reaction mixture to that of chemically prepared reference compound. [f]The absolute configurations of **8a**, **8f-h** and **8m** were determined by chiral HPLC using chemically synthesized authentic standards with known (*R*/*S*) and (*S*) configuration respectively. [g]The absolute configurations of **8c-e**, **8i-l** and **8n** were tentatively assigned the (*S*)-configuration based on analogy and according to chiral HPLC data.

Chemoenzymatic synthesis of chiral pyrazolidine-3-ones

Enantioselective conjugate addition of bisnucleophilic donors (such as hydrazines) to electron-poor acceptors provides convenient access to valuable small-ring heterocycles.[42] Encouraged by the exquisite stereoselectivity of the EDDS-lyase-catalyzed biotransformation accepting a broad range of arylamines (**7**, Table 1), we further questioned whether bisnucleophilic arylhydrazines (**9**, Table 2) could be processed as substrates by this enzyme in the amination of fumarate (**6**). The corresponding enzymatic products, *N*-(arylamino)aspartic acids (**10**), are not only valuable scaffolds in their own right, but they also could serve as chiral precursors for the preparation of synthetically challenging chiral pyrazolidine-3-ones (**11**) through an acid-catalyzed cyclization reaction (Table 2). Remarkably, phenylhydrazine (**9a**), as the first chosen potential bisnucleophilic substrate, was efficiently converted by EDDS lyase (0.1 mol%) to afford the single product *N*-(phenylamino) aspartic acid (**10a**), as ascertained by ^{1}H NMR in comparison with a chemically prepared authentic standard. In the enzymatic semipreparative synthesis (0.20 mmol scale) of compound **10a**, excellent conversion (94%) and good isolated yield (80%, 36 mg) were achieved (Table 2, entry 1). Note that it is necessary to perform the enzymatic reaction under anaerobic conditions, otherwise the substrate phenylhydrazine could be oxidized by molecular oxygen and thus lead to diminished conversion. Subsequently, the enzymatic product **10a** was cyclized smoothly under optimized conditions (1 M HCl, reflux for 3 h), affording the desired heterocycle 2-phenyl-5-carboxylpyrazolidin-3-one (**11a**, 71% isolated yield) without racemization of the potentially sensitive Cα stereogenic center (*ee* >99%, Table 2, entry 1). The chemoenzymatically prepared heterocycle **11a** was identified as the (*S*)-configured enantiomer by chiral HPLC analysis (Figure S15).

To further illustrate the synthetic usefulness of this chemoenzymatic method, we first determined that EDDS lyase has a broad substrate scope with respect to arylhydrazines (**9**), enabling the addition of various arylhydrazines to fumarate (Table S2). Pleasingly, several arylhydrazines with an *ortho*-substituent (*o*-fluoro, **9b**) or *meta*-substituent (such as *m*-fluoro, *m*-methyl, and *m*-chloro, **9c-e**) were efficiently converted by EDDS lyase giving the respective enzymatic products **10b-e** with excellent conversions (92-98%) and good isolated yields (76-85%, Table 2, entry 2-5). Notably, a number of arylhydrazines containing *para*-substituents, such as *p*-fluoro, *p*-chloro, *p*-bromo, *p*-cyano, *p*-methyl, and *p*-carboxyl (**9f-k**), were also well accepted by the enzyme giving the corresponding *N*-(arylamino) aspartic acids (**10f-k**) in high to excellent conversions (71-97%) and 52-89% isolated yields (Table 2, entry 6-11). However, *p*-(methoxyphenyl)hydrazine (**9l**) was not well accepted by EDDS lyase with unsatisfactory conversion (28%, Table 2, entry 12). Typically, arylhydrazines containing a strong electron-withdrawing group at the aromatic ring (namely *p*-nitro

or *p*-CF_3), or a bulky naphthalen-2-yl group, failed to undergo the EDDS-lyase-catalyzed hydroamination reaction (Table S2).

With the precious enzymatically prepared intermediates (**10b-k**) in hand, we subsequently performed the acid-catalyzed cyclization reaction to achieve the target 2,5-disubstituted pyrazolidin-3-one products. Remarkably, all the intermediates (**10b-k**) could be cyclized smoothly under the optimized conditions to provide the desired pyrazolidin-3-ones (**11b-k**) with good isolated yield (46-80%, Table 2, entry 2-11). Moreover, all the tested chemoenzymatic products (**11b-k**) were assigned the (*S*)-configuration, with excellent enantiomeric excess (*ee* >99%, Table 2, entry 2-11), using chiral HPLC analysis (Figures S16-S24). As such, we have established an efficient two-step chemoenzymatic route towards chiral 2-aryl-5-carboxylpyrazolidin-3-ones (**11a-k**) with good overall yields and excellent stereoselectivity (*ee* >99%).

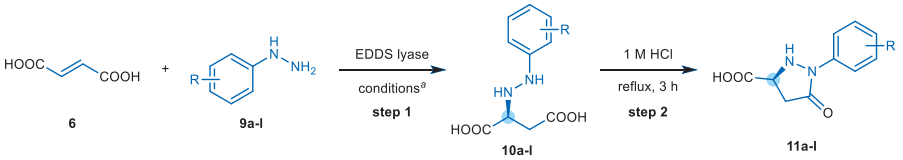
One-pot chemoenzymatic synthesis of chiral pyrazolidin-3-ones

Having established a stepwise chemoenzymatic route towards chiral pyrazolidin-3-ones (**11**), we sought to combine the EDDS lyase-catalyzed biotransformation and acid-catalyzed cyclization into one pot (Figure 2). In order to achieve high overall yield, as well as to effect the second cyclization step in the one-pot synthesis of pyrazolidin-3-ones (**11**), high conversion of the starting arylhydrazine substrates **9** in the first enzymatic step is required, preventing it from reacting with the intermediate **10** during the subsequent acid-promoted amidation step. Toward this end, the substrate phenylhydrazine (**9a**) that could be efficiently converted by EDDS lyase with 94% conversion was chosen for our initial investigation to provide the corresponding intermediate **10a** (Table 2 and Figure 2). Without any purification, intermediate **10a** was subjected to cyclization in the same pot, to which fuming hydrochloric acid (HCl) was added to adjust the final concentration of HCl to 1 M, providing full conversion for the second cyclization step after heating to reflux for 3 h. Pleasingly, the one-pot chemoenzymatically prepared product (*S*)-2-phenyl-5-carboxylpyrazolidin-3-one (**11a**) was isolated with good overall yield (68%) and excellent optical purity (*ee* >99%, Figure 2).

To further demonstrate the synthetic usefulness of this one-pot two-step chemoenzymatic strategy, we selected five other starting arylhydrazines (**9c-e** and **9h-i**, Table 2), which proved to be well accepted as substrates by EDDS lyase. The corresponding chemoenzymatically prepared (*S*)-pyrazolidin-3-one derivatives (**11c-e** and **11h-i**) were obtained with good overall isolated yields (61-70%) and excellent enantiopurity (*ee* >99% in all cases, Figure 2).

Therefore, this one-pot chemoenzymatic synthesis route provides a simplified practical procedure towards optically pure pyrazolidin-3-ones with higher overall isolated yields.

Table 2. Chemoenzymatic synthesis of chiral pyrazolidine-3-ones



			First enzymatic step		Second cyclization step		
Entry	Aryl hydrazine	R	Intermediate	Conv.[b] (Yield[c]) [%]	Product	Yield[c] [%]	*ee*[e] [%]
1	**9a**	H	**10a**	94 (80)	**11a**	71	>99 (*S*)[f]
2	**9b**	*o*-F	**10b**	98 (81)	**11b**	46	>99 (*S*)[g]
3	**9c**	*m*-F	**10c**	98 (83)	**11c**	73	>99 (*S*)[f]
4	**9d**	*m*-Me	**10d**	92 (76)	**11d**	62	>99 (*S*)[f]
5	**9e**	*m*-Cl	**10e**	98 (85)	**11e**	67	>99 (*S*)[f]
6	**9f**	*p*-F	**10f**	85 (52)	**11f**	59	>99 (*S*)[f]
7	**9g**	*p*-Cl	**10g**	80 (68)	**11g**	80	>99 (*S*)[f]
8	**9h**	*p*-Br	**10h**	91 (81)	**11h**	76	>99 (*S*)[f]
9	**9i**	*p*-CN	**10i**	92 (70)	**11i**	57	>99 (*S*)[g]
10	**9j**	*p*-Me	**10j**	71 (63)	**11j**	58	>99 (*S*)[f]
11	**9k**	*p*-CO_2H	**10k**	97 (89)	**11k**	69	n.d.[h]
12	**9l**	*p*-OMe	**10l**	28 (n.d.[d])	**11l**	–	–

[a]Conditions and reagents: fumaric acid (**6**, 50 mM), arylhydrazine substrates **9a-l** (10 mM) and purified EDDS lyase (0.1 mol% based on arylhydrazine) in degassed buffer (50 mM NaH_2PO_4/NaOH, pH 8.5) under argon atmosphere at room temperature (24 h for **10k**; 48 h for **10a**, **10c**, **10e**, and **10h-i**; 96 h for **10b**, **10d**, **10f-g**, **10j** and **10l**). [b]Conversions were determined by comparing ^{1}H NMR signals of substrates and corresponding products. [c]Isolated yield after purification. [d]Not determined owing to low conversion, the product formation was confirmed by comparison of ^{1}H NMR data of a crude reaction mixture to that of chemically prepared reference compound. [e]Enantiomeric excess (*ee*) was determined by HPLC on a chiral stationary phase using chemically synthesized racemic standards. [f]The absolute configurations of **11a**, **11c-h**, and **11j** were determined by chiral HPLC using authentic standards with known (*R*/*S*) and (*S*) configuration. [g]The absolute configurations of **11b** and **11i** were tentatively assigned the (*S*)-configuration based on analogy and according to chiral HPLC data. [h]The *ee* value was not determined.

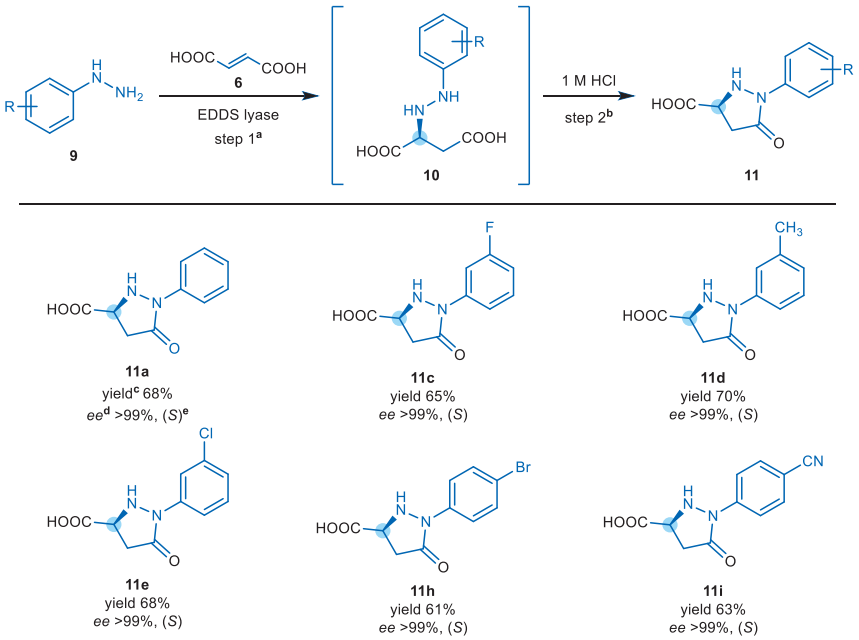


Figure 2. One-pot two-step chemoenzymatic synthesis of chiral pyrazolidin-3-ones. Reagents and reaction conditions: [a]arylhydrazine substrates **9** (10 mM), fumaric acid (**6**, 50 mM), and purified EDDS lyase (0.1 mol% based on arylhydrazine) in degassed buffer (50 mM NaH_2PO_4/NaOH, pH 8.5) under argon atmosphere at room temperature (48 h for **9a**, **9c**, **9e** and **9h-i**; 96 h for **9d**). [b]1 M HCl, reflux for 3 h under nitrogen atmosphere. [c]Isolated yield over two steps. [d]Enantiomeric excess (*ee*) was determined by HPLC on a chiral stationary phase using chemically synthesized racemic standards. [e]The absolute configuration of the one-pot chemoenzymatic products **11a**, **11c-e** and **11h** were determined by chiral HPLC analysis using authentic standards with known (*R*/*S*) and (*S*) configuration. The absolute configuration of product **11i** was tentatively assigned the (*S*)-configuration based on analogy and according to chiral HPLC data.

Discussion

In contrast to previously reported chemical synthesis strategies for preparation of enantio-enriched *N*-arylated α-amino acids, such as metal-catalyzed *N*-arylation[7-13] or hypervalent iodine chemistry[14], which mainly depend on extending the free amino group of the starting chiral α-amino acids (or their esters), our biocatalytic method starts with a prochiral α,β-unsaturated acid (fumarate) and creates the Cα-stereocentre of the target *N*-arylated amino acids in a single asymmetric step with excellent stereocontrol (Figure 1a). We demonstrated that EDDS lyase shows a remarkably broad substrate scope, enabling the addition of

a variety of aromatic amines (**7a-n**) to fumarate, yielding optically pure (*ee* >99%) (*S*)-*N*-arylated aspartic acids (**8a-n**) with high conversions and in good isolated yields (Table 1). Furthermore, we discovered that EDDS lyase can accept a wide range of arylhydrazines (**9a-k**) in the hydroamination of fumarate, yielding the corresponding *N*-(arylamino)-substituted aspartic acids (**10a-k**) with high conversions and in good isolated yields (Table 2). Subsequently, these enzymatic products (**11a-k**) could undergo a smooth acid-catalyzed cyclization to give the synthetically challenging chiral (*S*)-pyrazolidin-3-one derivatives **11a-k** with excellent enantiomeric excess (*ee* >99%, Table 2). In addition, we successfully combined the EDDS lyase-catalyzed biotransformation and acid-catalyzed cyclization into one pot, thus providing a rather simple two-step chemoenzymatic route for the rapid synthesis of optically pure pyrazolidin-3-ones with good overall isolated yields (Figure 2).

Enantioselective addition of ammonia or amines to appropriate α,β-unsaturated carboxylic acids catalyzed by carbon-nitrogen lyases represents an attractive strategy for the synthesis of chiral unnatural amino acids. This enzymatic strategy makes use of readily available prochiral α,β-unsaturated acids as starting substrates without a requirement for cofactor recycling, circumvents tedious steps of protecting or activating carboxylic groups, gives 100% theoretical yield, and normally provides high stereoselectivity under mild and potentially green reaction conditions. Several synthetically useful carbon-nitrogen lyases, such as aspartate ammonia lyases (DALs)[16,17,20,45], methylaspartate ammonia lyases (MALs)[16,17,21,46], phenylalanine ammonia lyases (PALs)[16,17] and phenylalanine aminomutases (PAMs)[16,17], were successfully used in the synthesis of optically pure unnatural α- or β-amino acids. However, with the exception of an engineered mutant of MAL (MAL-Q37A), which accepts various alkylamines as substrates in the addition to mesaconate[21], these enzymes display a rather limited nucleophile scope. In contrast, EDDS lyase has a very broad nucleophile scope, accepting a wide variety of structurally distinct amines for stereoselective addition to fumarate, providing enzymatic access to various aminocarboxylic acids including the natural products toxin A, aspergillomarasmine A and aspergillomarasmine B[23], *N*-cycloalkyl-substituted aspartic acids[24], as well as difficult *N*-arylated aspartic acid derivatives and substituted pyrazolidin-3-ones (this study). As such, EDDS lyase nicely complements the biocatalytic toolbox for the preparation of noncanonical amino acids. In future work, we will focus our attention on extending the electrophile scope of EDDS lyase by computational design and structure-guided protein engineering.

Methods

Enzymatic synthesis of (S)-*N*-arylated aspartic acids (8a-n)

Enzyme expression and purification were performed according to procedures described elsewhere (supplementary information).[23,44] The reaction mixture (15 mL) consisted of fumaric acid (50 mM) and an arylamine substrate (**7a-n**, 10 mM) in 50 mM NaH_2PO_4-NaOH buffer (pH 8.5) with 5% DMSO as cosolvent. The pH of the reaction mixture was adjusted to pH 8.5. The enzymatic reaction was started by addition of freshly purified EDDS lyase (0.05 mol%). The reaction mixture was then incubated at room temperature from 24 h to 72 h (Table 1). After completion of the reaction, the enzyme was inactivated by heating to 70 °C for 10 min. The progress of the enzymatic reaction was monitored by ^{1}H NMR spectroscopy by comparing signals of substrates and corresponding products.

The amino acid products were purified by cation-exchange chromatography. For a typical purification procedure, the precipitated enzyme was removed by filtration (pore diameter 0.45 μm). The filtrate was washed with ethyl acetate (10 mL x 3) to remove the remaining amines. The aqueous layer was acidified with 1 M HCl to pH=1 and loaded slowly onto a cation-exchange column (5 g of Dowex 50W X8 resin, 100-200 mesh), which was pre-treated with 2 M aqueous ammonia (5 column volumes), 1 M HCl (3 column volumes) and finally water (5 column volumes). The column was washed with water (3 column volumes) to remove the remaining fumaric acid and eluted with 2 M aqueous ammonia until the desired product was collected. The ninhydrin-positive fractions were collected, concentrated under vacuum and lyophilized to provide the desired products (**8a-n**) as ammonium salts.

One-pot chemoenzymatic synthesis of pyrazolidine-3-ones (11)

Step 1: The reaction mixture (20 mL) consisted of fumaric acid (50 mM) and an arylhydrazine substrate (**9**, 10 mM) in 50 mM NaH_2PO_4-NaOH degassed buffer (pH 8.5) under argon atmosphere. The pH of the reaction mixture was adjusted to pH 8.5. The enzymatic reaction was started by addition of freshly purified EDDS lyase (0.1 mol%). The reaction mixture was then incubated at room temperature from 48 h to 96 h (Figure 2). The progress of the enzymatic reaction was monitored by ^{1}H NMR spectroscopy by comparing signals of substrates and corresponding products. Without purification of the enzymatic product **10**, the reaction mixture was subjected to the next step immediately.

Step 2: To the stirred reaction mixture from the previous step was added 1.6 mL of fuming hydrochloric acid dropwise under cooling (ice-bath). After 5 min, the reaction mixture was heated to reflux for 3 h under nitrogen atmosphere. After completion of the reaction, the reaction mixture was allowed to cool down to room temperature. The reaction mixture was extracted with EtOAc (20 mL x 3), and washed with brine (30 mL). The solvent was evaporated to provide crude product **11**, which was purified by C18 column chromatography (5% to 50% CH_3CN in H_2O as the eluent).

Detailed experimental procedures

For detailed experimental procedures and characterization of compounds, see the supplementary information.

Data availability

All data are available from the corresponding author upon reasonable request. Correspondence and requests for materials should be addressed to G.J.P.

Acknowledgements

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Author contributions

H.F., A.P.L. and L.B. performed preparative biotransformations and product analysis. H.F. synthesized the reference compounds. H.F. and L.B. developed the one-pot chemoenzymatic cascade. H.F., J.Z. and P.G.T. performed chiral HPLC experiments. G.J.P. supervised scientific work. All authors contributed to writing the paper.

Notes

The authors declare no competing financial interests.

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Supplementary Information

Table of contents

I) General information

Fumaric acid, aromatic amines and hydrazines were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), TCI Europe N.V., Thermo Fisher Scientific (Geel, Belgium) or Fluorochem Co. (UK). Hypervalent iodine compounds and (*S*)-α-methylbenzylamine were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO). Solvents were purchased from Biosolve (Valkenswaard, The Netherlands) or Sigma-Aldrich Chemical Co. Ingredients for buffers and media were obtained from Duchefa Biochemie (Haarlem, The Netherlands) or Merck (Darmstadt, Germany). Dowex 50W X8 resin (hydrogen form, 100-200 mesh) was purchased from Sigma-Aldrich Chemical Co. Ni sepharose 6 fast flow resin and HiLoad 16/600 Superdex 200 pg column were purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Proteins were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under denaturing conditions on precast gels (NuPAGE™ 4–12% Bis-Tris protein gels). The gels were stained with Coomassie brilliant blue. High performance liquid chromatography (HPLC) was performed with a Shimadzu LC-10AT HPLC with a Shimadzu SPD-M10A diode array detector. NMR analysis was performed on a Brucker 500 MHz machine at the Drug Design laboratory of the University of Groningen. Chemical shifts (δ) are reported in parts per million (ppm). Electrospray ionization orbitrap high resolution mass spectrometry (HRMS) was performed by the Mass Spectrometry core facility of the University of Groningen.

II) Detailed experimental procedures

1. Expression and purification of EDDS lyase

E. coli TOP10 cells containing the pBADN (EDDS lyase-His) plasmid were collected from a LB/Ap plate, and used to inoculate LB/Ap medium (10 mL). The culture was used to inoculate fresh LB/Ap medium (1 L) after overnight incubation at 37°C. The cells were grown at 37°C for about 4 h until OD_{600} reached 0.8~1.0. Arabinose (0.05%, w/v) was added to induce the enzyme expression. Cultures were grown for 18 h at 20 °C with vigorous shaking. Cells were harvested by centrifugation and stored at -20 °C until further use. In a typical purification experiment, 4 g of wet cells (from 1 L culture) were suspended in lysis buffer (15 mL, 50 mM Tris-HCl, 300 mM NaCl, 20 mM imidazole, pH 8.0). Cells were disrupted by sonication for 4 x 40 s (with 5 min interval between each cycle) at a 60 W output. The unbroken cells and debris were removed by centrifugation. The supernatant was filtered through a pore filter (diameter 0.45 μm), and incubated with Ni sepharose resin (1 mL slurry in a small column) at 4 °C for 18 h, which had previously been equilibrated with lysis

buffer. The unbound proteins were eluted from the column by gravity flow. The column was washed with lysis buffer (15 mL x 2). Retained proteins were eluted with buffer A (5 mL, 50 mM Tris-HCl, 300 mM NaCl, 500 mM imidazole pH 8.0). Fractions were analyzed by SDS-PAGE on gels containing acrylamide (4 - 12%). Fractions containing EDDS lyase were combined and loaded onto a PD-10 Sephadex G-25 gelfiltration column, which was previously equilibrated with buffer B (25 mL, 50 mM NaH_2PO_4-NaOH buffer, pH 8.5). The column was eluted with buffer B (3.5 mL) and fractions were collected and analyzed by SDS-PAGE on gels containing acrylamide (4 - 12%). The purified enzyme was stored at -20 °C until further use.

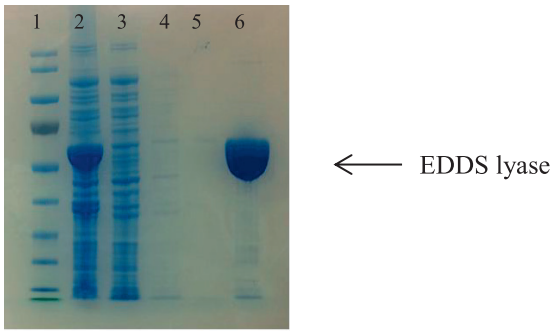
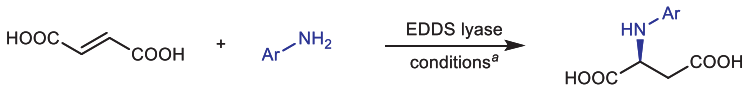


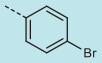
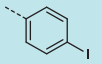
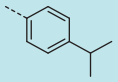
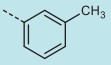
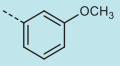
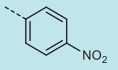
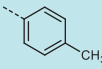
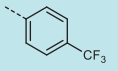
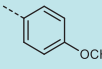
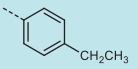
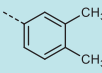
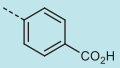
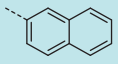
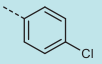
Figure S1. Purification of EDDS lyase by Ni-affinity chromatography. Lane 1: PageRuler™ prestained protein ladder (Thermo Scientific). Lane 2: cell free extract. Lane 3: unbound proteins in flow-through fractions. Lane 4 and 5: fractions from washing step with lysis buffer. Lane 6: fractions from elution step with buffer A. The molecular weight of EDDS lyase is about 56 kDa (including His-tag).

2. Screening aromatic amines and arylhydrazines as substrates for EDDS lyase

Table S1. Screening aromatic amines as substrates for EDDS lyase.

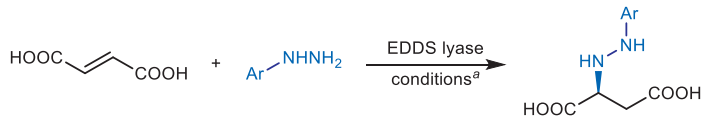


6 **7a-w** **8a-w**

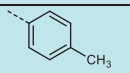
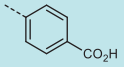
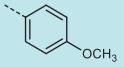
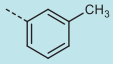
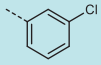
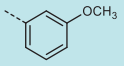
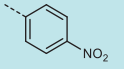
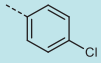
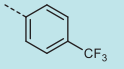
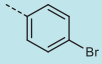
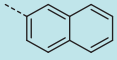
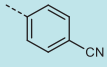
Entry	No.	Ar	Time [h]	Conv.[b] [%]	Entry	No.	Ar	Time [h]	Conv.[b] [%]
1	**7a**		48	91	13	**7m**		48	95
2	**7b**		72	6	14	**7n**		48	82
3	**7c**		48	91	15	**7o**		72	17
4	**7d**		24	97	16	**7p**		72	0
5	**7e**		48	87	17	**7q**		72	0
6	**7f**		24	92	18	**7r**		72	0
7	**7g**		48	75	19	**7s**		72	0
8	**7h**		48	92	20	**7t**		72	0
9	**7i**		48	76	21	**7u**		72	0
10	**7j**		72	90	22	**7v**		72	0
11	**7k**		24	95	23	**7w**		72	0
12	**7l**		48	96					

[a]Conditions and reagents: fumaric acid (**6**, 50 mM), aromatic amine substrates **7a-w** (10 mM) and purified EDDS lyase (0.05 mol% based on amine) in buffer (50 mM NaH_2PO_4/NaOH, pH 8.5), with 5% DMSO as cosolvent at room temperature. [b]Conversions were determined by comparing ^{1}H NMR signals of substrates and corresponding products. Product formation was confirmed by comparison of ^{1}H NMR data of the crude reaction mixture to those of chemically prepared reference compounds.

Table S2. Screening arylhydrazines as substrates for EDDS lyase.

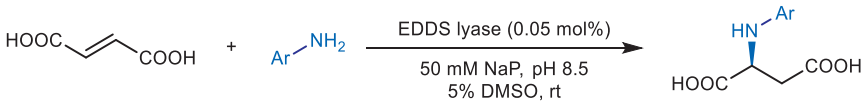


6 **9a-q** **10a-q**

Entry	No.	Ar	Time [h]	Conv.[b] [%]	Entry	No.	Ar	Time [h]	Conv.[b] [%]
1	**9a**		48	94	10	**9j**		96	71
2	**9b**		96	98	11	**9k**		24	97
3	**9c**		48	98	12	**9l**		96	28
4	**9d**		96	92	13	**9m**		72	0
5	**9e**		48	98	14	**9n**		72	0
6	**9f**		96	85	15	**9o**		72	0
7	**9g**		96	80	16	**9p**		72	0
8	**9h**		48	91	17	**9q**		72	0
9	**9i**		48	92					

[a]Conditions and reagents: fumaric acid (**6**, 50 mM), arylhydrazine substrates **9a-q** (10 mM) and purified EDDS lyase (0.1 mol% based on hydrazine) in degassed buffer (50 mM NaH_2PO_4/NaOH, pH 8.5) under argon atmosphere at room temperature. [b]Conversions were determined by comparing ^{1}H NMR signals of substrates and corresponding products. Product formation was confirmed by comparison of ^{1}H NMR data of the crude reaction mixture to those of chemically prepared reference compounds.

3. Enzymatic synthesis of (S)-*N*-aryl-substituted aspartic acids (8a-n)

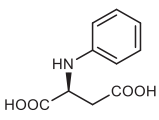


6 **7a-n** **8a-n**

General procedure: The reaction mixture (15 mL) consisted of fumaric acid (**6**, 50 mM) and an arylamine substrate (**7a-n**, 10 mM) in 50 mM NaH_2PO_4-NaOH buffer (pH 8.5) with 5% DMSO as cosolvent. The pH of the reaction mixture was adjusted to pH 8.5. The enzymatic reaction was started by addition of freshly purified EDDS lyase (0.05 mol%). The reaction mixture was then incubated at room temperature from 24 h to 72 h (Table S1). After completion of the reaction, the enzyme was inactivated by heating to 70 °C for 10 min. The progress of the enzymatic reaction was monitored by ^{1}H NMR spectroscopy by comparing signals of substrates and corresponding products.

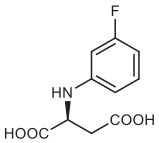
The amino acid products were purified by cation-exchange chromatography. For a typical purification procedure, the precipitated enzyme was removed by filtration (pore diameter 0.45 μm). The filtrate was washed with ethyl acetate (10 mL x 3) to remove the remaining amines. The aqueous layer was acidified with 1 M HCl to pH=1 and loaded onto a cation-exchange column (5 g of Dowex 50W X8 resin, 100-200 mesh), which was pretreated with 2 M aqueous ammonia (5 column volumes), 1 M HCl (3 column volumes) and water (5 column volumes). The column was washed with water (3 column volumes) to remove the remaining fumaric acid and eluted with 2 M aqueous ammonia until the desired product was collected. The ninhydrin-positive fractions were collected, concentrated under vacuum and lyophilized to provide the desired products as ammonium salts.

(*S*)-*N*-phenylaspartic acid (**8a**)



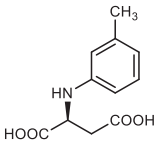
White solid. 25 mg (80% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.24 (t, J = 7.9 Hz, 2H), 6.81 (t, J = 7.3 Hz, 1H), 6.75 (d, J = 8.4 Hz, 2H), 4.12 (dd, J = 10.0, 3.9 Hz, 1H), 2.69 (dd, J = 15.0, 3.8 Hz, 1H), 2.45 (dd, J = 15.0, 10.0 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 181.1, 179.2, 147.2, 129.5 (2C), 118.8, 114.5 (2C), 57.8, 40.7. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}NO_4$ $[M+H]^+$: 210.0761, found: 210.0762. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R = 8.9 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S2).

(*S*)-*N*-(3-fluorophenyl)aspartic acid (**8c**)



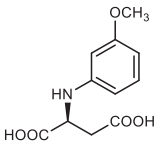
White solid. 18 mg (53% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.18 (q, J = 7.7 Hz, 1H), 6.51 – 6.43 (m, 3H), 4.11 (dd, J = 10.0, 3.9 Hz, 1H), 2.74 (dd, J = 15.2, 3.9 Hz, 1H), 2.49 (dd, J = 15.2, 10.0 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.8, 178.7, 163.8 (d, J = 240.7 Hz), 149.6 (d, J = 11.3 Hz), 130.7 (d, J = 10.1 Hz), 109.81, 104.4 (d, J = 21.4 Hz), 100.5 (d, J = 25.2 Hz), 57.3, 40.5. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4F$ $[M+H]^+$: 228.0667, found: 228.0664. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R = 7.7 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S3).

(*S*)-*N*-(3-methylphenyl)aspartic acid (**8d**)



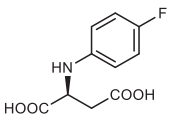
White solid. 28 mg (84% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.17 (t, J = 7.8 Hz, 1H), 6.73 (d, J = 7.5 Hz, 1H), 6.67 (s, 1H), 6.63 (d, J = 8.2 Hz, 1H), 4.14 (dd, J = 9.7, 3.9 Hz, 1H), 2.71 (dd, J = 15.3, 4.0 Hz, 1H), 2.50 (dd, J = 15.3, 9.5 Hz, 1H), 2.27 (s, 3H); ^{13}C NMR (126 MHz, D_2O): δ 180.5, 179.0, 146.6, 139.9, 129.5, 120.2, 115.6, 112.2, 58.2, 40.4, 20.6. HRMS (ESI$^+$): calcd. for $C_{11}H_{14}NO_4$ $[M+H]^+$: 224.0917, found: 224.0917. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R = 10.6 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S4).

(*S*)-*N*-(3-methoxyphenyl)aspartic acid (**8e**)



White solid. 19 mg (53% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.16 (t, J = 8.1 Hz, 1H), 6.39 (dd, J = 12.9, 8.3 Hz, 2H), 6.33 (s, 1H), 4.12 (dd, J = 9.9, 3.8 Hz, 1H), 3.78 (s, 3H), 2.71 (dd, J = 15.0, 3.8 Hz, 1H), 2.46 (dd, J = 15.1, 9.9 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.9, 178.9, 159.9, 148.9, 130.4, 107.4, 104.2, 99.7, 57.6, 55.1, 40.5. HRMS (ESI$^+$): calcd. for $C_{11}H_{14}NO_5$ $[M+H]^+$: 240.0866, found: 240.0866. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R = 10.7 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S5).

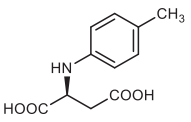
(*S*)-*N*-(4-fluorophenyl)aspartic acid (**8f**)



White solid. 29 mg (85% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.01 (t, J = 8.9 Hz, 2H), 6.80 – 6.77 (m, 2H), 4.10 (dd, J = 9.8, 3.9 Hz, 1H), 2.71 (dd, J = 15.2, 3.9 Hz, 1H), 2.48 (dd, J = 15.1, 9.8 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.3, 178.9, 156.9 (d, J = 234.4 Hz), 142.8, 116.5 (d, J = 7.6 Hz,

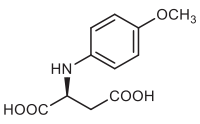
2C), 115.8, (d, J = 21.4 Hz, 2C), 58.8, 40.3. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4F$ $[M+H]^+$: 228.0667, found: 228.0667. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R = 6.8 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S6).

(*S*)-*N*-(4-methylphenyl)aspartic acid (**8g**)



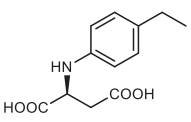
White solid. 19 mg (57% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.15 (d, J = 8.1 Hz, 2H), 6.89 (d, J = 8.5 Hz, 2H), 4.10 (dd, J = 8.6, 4.2 Hz, 1H), 2.65 (dd, J = 16.0, 4.2 Hz, 1H), 2.54 (dd, J = 16.0, 8.7 Hz, 1H), 2.23 (s, 3H); ^{13}C NMR (126 MHz, D_2O): δ 178.3, 178.2, 140.2, 132.6, 130.0 (2C), 117.6 (2C), 59.9, 38.4, 19.6. HRMS (ESI$^+$): calcd. for $C_{11}H_{14}NO_4$ $[M+H]^+$: 224.0917, found: 224.0922. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R = 10.0 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S7).

(*S*)-*N*-(4-methoxyphenyl)aspartic acid (**8h**)



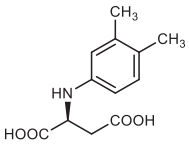
Light pink solid. 27 mg (75% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.10 (d, J = 8.6 Hz, 2H), 7.01 (d, J = 8.9 Hz, 2H), 4.13 (dd, J = 8.3, 4.2 Hz, 1H), 3.83 (s, 3H), 2.70 (dd, J = 16.3, 4.2 Hz, 1H), 2.61 (dd, J = 16.3, 8.3 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 178.9, 178.7, 154.2, 137.8, 118.6 (2C), 115.3 (2C), 60.2, 55.9, 39.2. HRMS (ESI$^+$): calcd. for $C_{11}H_{14}NO_5$ $[M+H]^+$: 240.0866, found: 240.0863. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R = 8.0 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S8).

(*S*)-*N*-(4-ethylphenyl)aspartic acid (**8i**)



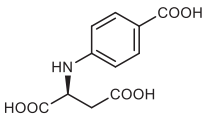
Light yellow solid. 19 mg (53% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.17 (d, J = 8.1 Hz, 2H), 6.81 (d, J = 8.1 Hz, 2H), 4.12 (dd, J = 9.5, 4.0 Hz, 1H), 2.68 (dd, J = 15.3, 4.0 Hz, 1H), 2.55 (q, J = 7.7 Hz, 2H), 2.49 (dd, J = 15.4, 9.4 Hz, 1H), 1.15 (t, J = 7.7 Hz, 3H); ^{13}C NMR (126 MHz, D_2O): δ 180.4, 179.1, 143.9, 136.4, 128.8 (2C), 115.8 (2C), 58.8, 40.3, 27.4, 15.3. HRMS (ESI$^+$): calcd. for $C_{12}H_{16}NO_4$ $[M+H]^+$: 238.1074, found: 238.1074. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R = 14.6 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S9).

(*S*)-*N*-(3,4-dimethylphenyl)aspartic acid (**8j**)



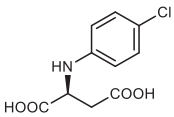
Light yellow solid. 25 mg (70% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.11 (d, J = 8.1 Hz, 1H), 6.83 (s, 1H), 6.75 (dd, J = 8.0, 2.4 Hz, 1H), 4.11 (dd, J = 8.6, 4.2 Hz, 1H), 2.66 (dd, J = 16.0, 4.3 Hz, 1H), 2.55 (dd, J = 16.0, 8.6 Hz, 1H), 2.21 (s, 3H), 2.17 (s, 3H); ^{13}C NMR (126 MHz, D_2O): δ 178.5 (2C), 141.1, 138.5, 131.0, 130.5, 118.6, 114.8, 59.8, 38.8, 19.0, 18.0. HRMS (ESI$^+$): calcd. for $C_{12}H_{16}NO_4$ [M+H]$^+$: 238.1074, found: 238.1073. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4I$ [M+H]$^+$: 335.9727, found: 335.9729. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R = 15.0 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S10).

(*S*)-*N*-(4-carboxyphenyl)aspartic acid (**8k**)



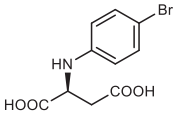
White solid. 13 mg (34% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.79 (d, J = 8.5 Hz, 2H), 6.69 (d, J = 8.4 Hz, 2H), 4.22 (dd, J = 9.0, 3.4 Hz, 1H), 2.81 (dd, J = 15.6, 3.5 Hz, 1H), 2.60 (dd, J = 15.5, 9.1 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.7, 178.8, 174.5, 151.0, 131.2, 131.0, 122.6, 112.3, 112.2, 56.4, 40.4. HRMS (ESI$^+$): calcd. for $C_{11}H_{12}NO_6$ [M+H]$^+$: 254.0659, found: 254.0656. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R = 4.5 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S11).

(*S*)-*N*-(4-chlorophenyl)aspartic acid (**8l**)



White solid. 23 mg (63% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.21 (d, J = 8.8 Hz, 2H), 6.70 (d, J = 8.9 Hz, 2H), 4.11 (dd, J = 10.0, 4.0 Hz, 1H), 2.72 (dd, J = 15.2, 4.0 Hz, 1H), 2.48 (dd, J = 15.2, 9.9 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.7, 178.8, 146.2, 129.0 (2C), 122.5, 115.5 (2C), 57.6, 40.4. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4Cl$ [M+H]$^+$: 244.0371, found: 244.0373. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R = 8.3 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S12).

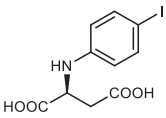
(*S*)-*N*-(4-bromophenyl)aspartic acid (**8m**)



White solid. 30 mg (69% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.35 (d, J = 8.6 Hz, 2H), 6.65 (d, J = 8.5 Hz, 2H), 4.10 (dd, J = 9.9, 4.0 Hz, 1H), 2.74 (dd, J = 15.2, 4.0 Hz, 1H), 2.49 (dd, J = 15.2, 9.8 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.7, 178.8, 146.7, 131.9 (2C), 115.9 (2C), 109.5, 57.5, 40.4.

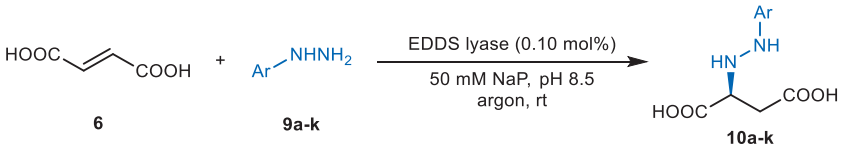
HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4Br$ $[M+H]^+$: 287.9866, found: 287.9864. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R = 9.3 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S13).

(*S*)-*N*-(4-iodophenyl)aspartic acid (**8n**)



White solid. 26 mg (52% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.52 (d, J = 8.5 Hz, 2H), 6.55 (d, J = 8.3 Hz, 2H), 4.10 (dd, J = 10.0, 3.9 Hz, 1H), 2.74 (dd, J = 15.3, 3.9 Hz, 1H), 2.49 (dd, J = 15.2, 9.9 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.6, 178.7, 147.4, 137.9 (2C), 116.4 (2C), 78.6, 57.2, 40.4. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4I$ $[M+H]^+$: 335.9727, found: 335.9729. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R = 14.8 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S14).

4. Enzymatic synthesis of (S)-*N*-(arylamino)aspartic acids (10a-k)

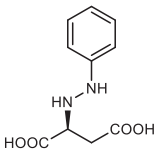


General procedure: The reaction mixture (20 mL) consisted of fumaric acid (50 mM) and arylhydrazines (**9a-k**, 10 mM) in 50 mM NaH_2PO_4-NaOH degassed buffer (pH 8.5) under argon atmosphere. The pH of the reaction mixture was adjusted to pH 8.5. The enzymatic reaction was started by addition of freshly purified EDDS lyase (0.1 mol%). The reaction mixture was then incubated at room temperature from 24 h to 96 h (Table S2). After completion of the reaction, the enzyme was inactivated by heating to 70 °C for 10 min. The progress of the enzymatic reaction was monitored by ^{1}H NMR spectroscopy by comparing signals of substrates and corresponding products.

The products were purified by cation-exchange chromatography. For a typical purification procedure, the precipitated enzyme was removed by filtration (pore diameter 0.45 μm). The filtrate was washed with ethyl acetate (15 mL x 3) to remove the remaining hydrazines. The aqueous layer was acidified with 1 M HCl to pH=1 and loaded onto a cation-exchange column (5 g of Dowex 50W X8 resin, 100-200 mesh), which was pretreated with 2 M aqueous ammonia (5 column volumes), 1 M HCl (3 column volumes) and water (5 column volumes). The column was washed with water (3 column volumes) to remove the remaining

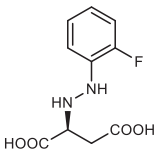
fumaric acid and eluted with 2 M aqueous ammonia until the desired product was collected. The ninhydrin-positive fractions were collected, concentrated under vacuum and lyophilized to provide the desired products as ammonium salts.

(*S*)-*N*-(phenylamino)aspartic acid (**10a**)



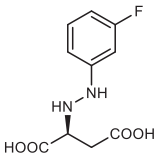
White solid. 36 mg (80% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.33 (t, J = 7.8 Hz, 2H), 7.06 (d, J = 8.2 Hz, 2H), 7.01 (t, J = 7.5 Hz, 1H), 3.83 (dd, J = 9.3, 4.2 Hz, 1H), 2.70 (dd, J = 16.1, 4.2 Hz, 1H), 2.50 (dd, J = 16.1, 9.3 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 178.7, 178.3, 146.2, 129.4 (2C), 121.6, 115.7 (2C), 61.0, 38.0. HRMS (ESI$^+$): calcd. for $C_{10}H_{13}N_2O_4$ $[M+H]^+$: 225.0870, found: 225.0869.

(*S*)-*N*-(2-fluorophenylamino)aspartic acid (**10b**)



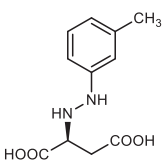
Light yellow solid. 39 mg (81% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.32 (td, J = 8.3, 1.7 Hz, 1H), 7.17 – 7.09 (m, 2H), 6.96 – 6.91 (m, 1H), 3.77 (dd, J = 9.8, 4.0 Hz, 1H), 2.67 (dd, J = 15.7, 4.0 Hz, 1H), 2.43 (dd, J = 15.7, 9.8 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 179.2, 178.9, 152.1 (d, J = 239.4 Hz), 134.7, 124.8, 121.3, 117.2, 115.1 (d, J = 17.6 Hz), 61.3, 38.5. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}N_2O_4F$ $[M+H]^+$: 243.0776, found: 243.0776.

(*S*)-*N*-(3-fluorophenylamino)aspartic acid (**10c**)



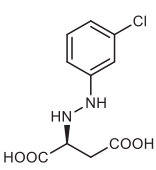
Light yellow solid. 40 mg (83% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.22 (q, J = 8.1 Hz, 1H), 6.82 – 6.74 (m, 2H), 6.59 (td, J = 8.6, 2.6 Hz, 1H), 3.72 (dd, J = 9.9, 4.0 Hz, 1H), 2.62 (dd, J = 15.4, 4.1 Hz, 1H), 2.37 (dd, J = 15.4, 9.9 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 179.9, 179.2, 163.6 (d, J = 241.9 Hz), 149.8 (d, J = 11.3 Hz), 130.5 (d, J = 12.6 Hz), 110.1 (d, J = 16.4 Hz), 106.6, 101.4, 61.6, 38.9. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}N_2O_4F$ $[M+H]^+$: 243.0776, found: 243.0775.

(*S*)-*N*-(3-methylphenylamino)aspartic acid (**10d**)



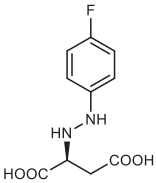
Orange solid. 36 mg (76% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.22 (t, J = 7.8 Hz, 1H), 6.91 (s, 1H), 6.87 – 6.84 (m, 2H), 3.83 (dd, J = 9.1, 4.2 Hz, 1H), 2.71 (dd, J = 16.1, 4.2 Hz, 1H), 2.51 (dd, J = 16.1, 9.1 Hz, 1H), 2.28 (s, 3H); ^{13}C NMR (126 MHz, D_2O): δ 178.4, 177.5, 145.7, 139.9, 129.4, 122.7, 116.4, 113.0, 60.8, 37.4, 20.5. HRMS (ESI$^+$): calcd. for $C_{11}H_{15}N_2O_4$ $[M+H]^+$: 239.1026, found: 239.1028.

(*S*)-*N*-(3-chlorophenylamino)aspartic acid (**10e**)



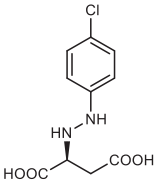
Light yellow solid. 44 mg (85% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.22 (t, J = 8.0 Hz, 1H), 7.08 (t, J = 2.1 Hz, 1H), 6.90 (d, J = 8.1 Hz, 2H), 3.77 (dd, J = 9.4, 4.2 Hz, 1H), 2.68 (dd, J = 15.8, 4.2 Hz, 1H), 2.45 (dd, J = 15.8, 9.4 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 178.8, 178.5, 148.7, 134.4, 130.5, 120.2, 114.3, 113.0, 61.2, 38.0. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}N_2O_4Cl$ [M+H]$^+$: 259.0480, found: 259.0480.

(*S*)-*N*-(4-fluorophenylamino)aspartic acid (**10f**)



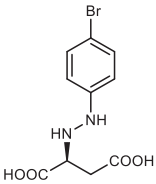
Light yellow solid. 25 mg (52% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.07 – 7.08 (m, 4H), 3.84 (dd, J = 9.2, 4.2 Hz, 1H), 2.73 (dd, J = 16.3, 4.2 Hz, 1H), 2.52 (dd, J = 16.3, 9.2 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 178.2, 177.1, 158.4 (d, J = 238.1 Hz), 141.4, 118.4 (d, J = 8.8 Hz, 2C), 115.8 (d, J = 22.7 Hz, 2C), 60.7, 37.1. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}N_2O_4F$ [M+H]$^+$: 243.0776, found: 243.0777.

(*S*)-*N*-(4-chlorophenylamino)aspartic acid (**10g**)



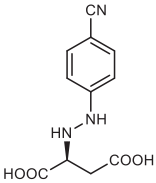
Light yellow solid. 35 mg (68% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.27 (d, J = 8.8 Hz, 2H), 6.99 (d, J = 8.9 Hz, 2H), 3.76 (dd, J = 9.4, 4.2 Hz, 1H), 2.67 (dd, J = 15.8, 4.2 Hz, 1H), 2.44 (dd, J = 15.8, 9.5 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 178.6, 178.5, 145.5, 129.0 (2C), 125.2, 116.6 (2C), 61.1, 38.0. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}N_2O_4Cl$ [M+H]$^+$: 259.0480, found: 259.0480.

(*S*)-*N*-(4-bromophenylamino)aspartic acid (**10h**)



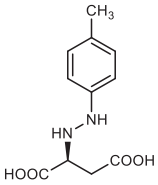
Light yellow solid. 49 mg (81% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.42 (d, J = 8.8 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 3.78 (dd, J = 9.4, 4.2 Hz, 1H), 2.69 (dd, J = 15.9, 4.2 Hz, 1H), 2.46 (dd, J = 15.8, 9.4 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 178.5, 178.4, 146.0, 131.9 (2C), 116.9 (2C), 112.4, 61.1, 37.8. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}N_2O_4Br$ [M+H]$^+$: 302.9975, found: 302.9974.

(*S*)-*N*-(4-cyanophenylamino)aspartic acid (**10i**)



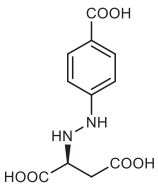
Yellow solid. 35 mg (70% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.48 (d, J = 8.8 Hz, 2H), 6.92 (d, J = 8.9 Hz, 2H), 3.68 (dd, J = 9.7, 4.2 Hz, 1H), 2.62 (dd, J = 15.5, 4.2 Hz, 1H), 2.37 (dd, J = 15.5, 9.8 Hz, 1H); ^{13}C NMR (126 MHz, D_2O) δ 179.7, 178.7, 152.6, 133.9, 133.8, 121.4, 112.5, 112.4, 98.5, 61.8, 38.4. HRMS (ESI$^+$): calcd. for $C_{11}H_{12}N_3O_4$ [M+H]$^+$: 250.0822, found: 250.0818.

(*S*)-*N*-(4-methylphenylamino)aspartic acid (**10j**)



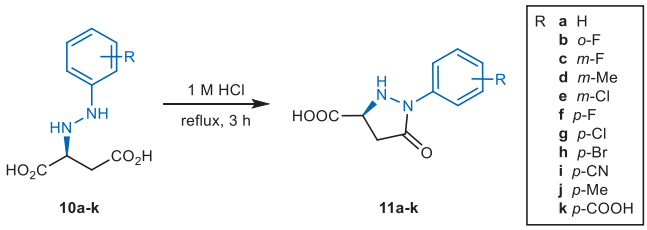
Orange solid. 30 mg (63% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.15 (d, J = 8.0 Hz, 2H), 6.96 (d, J = 8.2 Hz, 2H), 3.74 (dd, J = 9.5, 4.1 Hz, 1H), 2.63 (dd, J = 15.6, 4.1 Hz, 1H), 2.41 (dd, J = 15.6, 9.6 Hz, 1H), 2.25 (s, 3H); ^{13}C NMR (126 MHz, D_2O): δ 179.3, 179.2, 144.2, 131.2, 129.7 (2C), 116.0 (2C), 60.9, 38.8, 19.5. HRMS (ESI$^+$): calcd. for $C_{11}H_{15}N_2O_4$ $[M+H]^+$: 239.1026, found: 239.1025.

(*S*)-*N*-(4-carboxyphenylamino)aspartic acid (**10k**)



Light yellow solid. 48 mg (89% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.78 (d, J = 8.8 Hz, 2H), 6.99 (d, J = 8.7 Hz, 2H), 3.77 (dd, J = 9.7, 4.1 Hz, 1H), 2.66 (dd, J = 15.5, 4.1 Hz, 1H), 2.42 (dd, J = 15.5, 9.7 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 179.6, 179.0, 175.1, 150.8, 130.8, 130.6, 126.2, 112.9 (2C), 61.7, 38.6. HRMS (ESI$^+$): calcd. for $C_{11}H_{12}N_2O_6$ $[M+H]^+$: 269.0768, found: 269.0768.

5. Synthesis of (S)-2-aryl-5-carboxylpyrazolidin-3-ones (11a-k)

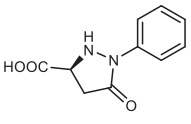


General procedure. The enzymatic products *N*-arylamino-substituted aspartic acids (**10a**, 32 mg, 0.14 mmol; **10b**, 35 mg, 0.14 mmol; **10c**, 34 mg, 0.14 mmol; **10d**, 33 mg, 0.14 mmol; **10e**, 37 mg, 0.14 mmol; **10f**, 20 mg, 0.08 mmol; **10g**, 35 mg, 0.14 mmol; **10h**, 45 mg, 0.15 mmol; **10i**, 32 mg, 0.13 mmol; **10j**, 28 mg, 0.12 mmol; **10k**, 45 mg, 0.17 mmol) was dissolved in 1 M HCl aqueous solution (3 mL). The reaction mixture was heated to reflux for 3 h under nitrogen atmosphere. After completion of the reaction, the reaction mixture was allowed to cool down to room temperature and then kept in an ice-bath for 30 min

For compounds **11a**, **11c-e**, **11g-h** and **11j-k**, the desired product was precipitated from the reaction mixture. The product was filtered off, washed with cold water (2 mL) and dried under vacuum overnight. For compound **11f**, the reaction mixture was extracted with EtOAc (5 mL x 3). The combined organic layers were washed with brine (10 mL), dried over

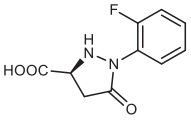
Na_2SO_4 and evaporated to provide pure product. For compound **11b** and **11i**, the reaction mixture was extracted with EtOAc (5 mL x 3). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4 and evaporated to provide crude product, which was further purified via C18 column chromatography (5% to 50% CH_3CN in H_2O as the eluent).

(*S*)-2-phenyl-5-carboxylpyrazolidin-3-one (**11a**)



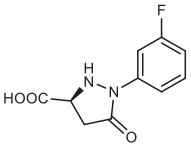
Light yellow solid. 21 mg (71% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.00 (brs, 1H), 7.79 – 7.77 (m, 2H), 7.37 – 7.34 (m, 2H), 7.09 (tt, J = 7.2, 1.2 Hz, 1H), 6.55 (brs, 1H), 4.25 (dd, J = 8.5, 5.9 Hz, 1H), 2.99 (dd, J = 16.4, 8.6 Hz, 1H), 2.77 (dd, J = 16.4, 5.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.3, 170.1, 139.0, 128.5 (2C), 123.6, 117.9, 117.8, 54.8, 37.3. The NMR data are in agreement with published data.[1] HRMS (ESI$^+$): calcd. for $C_{10}H_{11}N_2O_3$ [M+H]$^+$: 207.0764, found: 207.0763. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R = 9.1 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S15).

(*S*)-2-(2-fluorophenyl)-5-carboxylpyrazolidin-3-one (**11b**)



White solid. 15 mg (46% yield). ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.55 – 7.52 (m, 1H), 7.38 – 7.34 (m, 1H), 7.24 – 7.20 (m, 2H), 4.38 (t, J = 7.3 Hz, 1H), 3.09 (dd, J = 16.7, 8.8 Hz, 1H), 2.91 (dd, J = 16.7, 5.8 Hz, 1H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 172.6, 171.4, 156.8 (d, J = 252.0 Hz), 129.2 (d, J = 7.6 Hz), 127.43, 125.1 (d, J = 12.6 Hz), 124.2 (d, J = 3.8 Hz), 116.0 (d, J = 18.9 Hz), 56.4, 35.6. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3F$ [M+H]$^+$: 225.0670, found: 225.0670. Chiral HPLC conditions D: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (15%, v/v, 0.1% TFA) as mobile phase with a flow rate of 0.25 mL/min, rt, UV detection at 260 nm, t_R = 14.1 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S16).

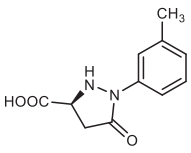
(*S*)-2-(3-fluorophenyl)-5-carboxylpyrazolidin-3-one (**11c**)



Light yellow solid. 23 mg (73% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.03 (brs, 1H), 7.64 – 7.60 (m, 2H), 7.39 (q, J = 8.3 Hz, 1H), 6.93 (td, J = 8.4, 2.5 Hz, 1H), 6.60 (brs, 1H), 4.26 (dd, J = 8.5, 5.8 Hz, 1H), 3.01 (dd, J = 16.6, 8.5 Hz, 1H), 2.78 (dd, J = 16.5, 5.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.2, 170.7, 162.0 (d, J = 241.9 Hz), 140.5 (d, J = 11.3 Hz), 130.3 (d, J = 10.1 Hz), 113.5 (d, J = 17.6 Hz), 109.9 (d, J = 34.0 Hz), 104.4 (d, J = 26.5 Hz), 54.8, 37.3. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3F$ [M+H]$^+$: 225.0670, found: 225.0669. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/

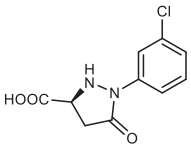
H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R = 14.5 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S17).

(*S*)-2-(3-methylphenyl)-5-carboxylpyrazolidin-3-one (**11d**)



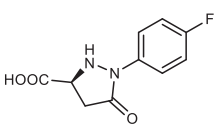
Light yellow solid. 19 mg (62% yield). ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.56 (s, 1H), 7.52 (dd, J = 8.2, 2.1 Hz, 1H), 7.16 (t, J = 7.9 Hz, 1H), 6.91 (d, J = 7.5 Hz, 1H), 4.24 (dd, J = 8.7, 6.7 Hz, 1H), 3.00 (dd, J = 16.6, 8.7 Hz, 1H), 2.86 (dd, J = 16.6, 6.7 Hz, 1H), 2.27 (s, 3H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 173.9, 171.8, 139.7, 139.6, 129.4, 126.6, 121.0, 117.6, 56.4, 38.6, 21.6. HRMS (ESI$^+$): calcd. for $C_{11}H_{13}N_2O_3$ [M+H]$^+$: 221.0921, found: 221.0922. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R = 12.0 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S18)

(*S*)-2-(3-chlorophenyl)-5-carboxylpyrazolidin-3-one (**11e**)



Light yellow solid. 23 mg (67% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.04 (brs, 1H), 7.84 (t, J = 2.1 Hz, 1H), 7.75 – 7.73 (m, 1H), 7.39 (t, J = 8.2 Hz, 1H), 7.16 – 7.14 (m, 1H), 6.60 (brs, 1H), 4.26 (dd, J = 8.5, 5.8 Hz, 1H), 3.01 (dd, J = 16.5, 8.6 Hz, 1H), 2.77 (dd, J = 16.6, 5.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.2, 170.8, 140.2, 133.0, 130.4, 123.2, 117.2, 116.2, 54.8, 37.3. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3Cl$ [M+H]$^+$: 241.0374, found: 241.0373. Chiral HPLC conditions E: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 1.0 mL/min, rt, UV detection at 260 nm, t_R = 13.3 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S19).

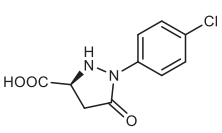
(*S*)-2-(4-fluorophenyl)-5-carboxylpyrazolidin-3-one (**11f**)



Light yellow solid. 11 mg (59% yield). ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.82 – 7.80 (m, 2H), 7.09 (t, J = 8.8 Hz, 2H), 4.32 (dd, J = 8.7, 6.6 Hz, 1H), 3.08 (dd, J = 16.6, 8.7 Hz, 1H), 2.93 (dd, J = 16.6, 6.6 Hz, 1H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 173.9, 171.9, 161.0 (d, J = 243.2 Hz), 136.1 (d, J = 2.5 Hz), 122.3 (d, J = 8.8 Hz, 2C), 116.1 (d, J = 22.7 Hz, 2C), 56.4, 38.5. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3F$ [M+H]$^+$: 225.0670, found: 225.0669. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at

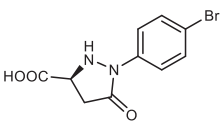
260 nm, t_R = 11.2 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S20).

(*S*)-2-(4-chlorophenyl)-5-carboxylpyrazolidin-3-one (**11g**)



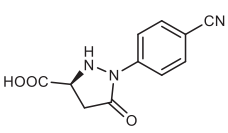
Light yellow solid. 26 mg (80% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.01 (brs, 1H), 7.79 (d, J = 8.9 Hz, 2H), 7.41 (d, J = 8.8 Hz, 2H), 6.58 (brs, 1H), 4.25 (dd, J = 8.4, 6.0 Hz, 1H), 2.99 (dd, J = 16.5, 8.5 Hz, 1H), 2.76 (dd, J = 16.6, 5.9 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.2, 170.4, 137.9, 128.5, 128.4, 127.2, 119.4, 119.3, 54.8, 37.2. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3Cl$ [M+H]$^+$: 241.0374, found: 241.0375. Chiral HPLC conditions F: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (25%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 1.0 mL/min, rt, UV detection at 260 nm, t_R = 9.8 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S21).

(*S*)-2-(4-bromophenyl)-5-carboxylpyrazolidin-3-one (**11h**)



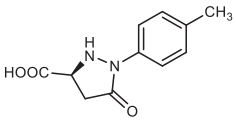
Light yellow solid. 32 mg (76% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.01 (brs, 1H), 7.74 (d, J = 9.0 Hz, 2H), 7.54 (d, J = 8.9 Hz, 2H), 6.58 (brs, 1H), 4.25 (dd, J = 8.5, 5.9 Hz, 1H), 2.98 (dd, J = 16.5, 8.5 Hz, 1H), 2.76 (dd, J = 16.5, 5.9 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.2, 170.4, 138.3, 131.4, 131.3, 119.8, 119.6, 115.2, 54.8, 37.2. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3Br$ [M+H]$^+$: 284.9869, found: 284.9868. Chiral HPLC conditions F: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (25%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 1.0 mL/min, rt, UV detection at 260 nm, t_R = 14.5 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S22).

(*S*)-2-(4-cyanophenyl)-5-carboxylpyrazolidin-3-one (**11i**)



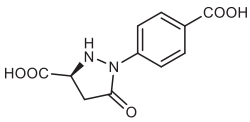
Yellow solid. 17 mg (57% yield). ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.05 (d, J = 8.6 Hz, 2H), 7.70 (d, J = 8.7 Hz, 2H), 4.29 (t, J = 7.0 Hz, 1H), 3.07 (dd, J = 16.8, 8.2 Hz, 1H), 2.96 (dd, J = 16.8, 7.3 Hz, 1H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 173.6, 173.1, 143.9, 134.0, 133.9, 119.8, 119.5 (2C), 107.7, 56.5, 38.8. HRMS (ESI$^+$): calcd. for $C_{11}H_{10}N_3O_3$ [M+H]$^+$: 232.0717, found: 232.0718. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R = 19.0 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S23).

(*S*)-2-(4-methylphenyl)-5-carboxylpyrazolidin-3-one (**11j**)



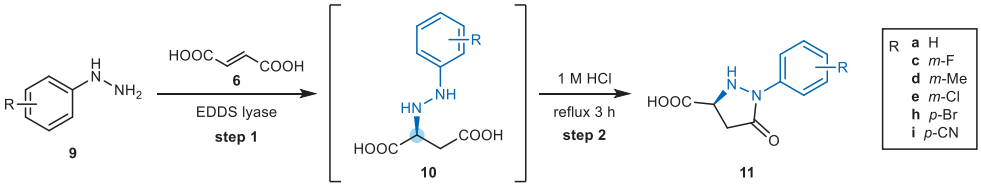
Orange solid. 15 mg (58% yield). ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.65 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 8.3 Hz, 2H), 4.31 (dd, J = 8.8, 6.7 Hz, 1H), 3.07 (dd, J = 16.6, 8.8 Hz, 1H), 2.93 (dd, J = 16.6, 6.7 Hz, 1H), 2.31 (s, 3H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 174.0, 171.6, 137.3, 135.8, 130.1 (2C), 120.6 (2C), 56.4, 38.6, 20.9. HRMS (ESI$^+$): calcd. for $C_{11}H_{13}N_2O_3$ [M+H]$^+$: 221.0921, found: 221.0921. Chiral HPLC conditions G: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (25%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R = 11.1 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S24).

(*S*)-2-(4-carboxylphenyl)-5-carboxylpyrazolidin-3-one (**11k**)



Yellow solid. 29 mg (69% yield). ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.01 (d, J = 9.0 Hz, 2H), 7.96 (d, J = 9.1 Hz, 2H), 4.35 (dd, J = 8.6, 6.7 Hz, 1H), 3.11 (dd, J = 16.7, 8.6 Hz, 1H), 2.96 (dd, J = 16.7, 6.7 Hz, 1H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 173.8, 172.8, 169.4, 143.9, 131.4 (2C), 127.2, 118.9 (2C), 56.4, 38.8. HRMS (ESI$^+$): calcd. for $C_{11}H_{11}N_2O_5$ [M+H]$^+$: 251.0662, found: 251.0662.

6. One-pot chemoenzymatic synthesis of chiral pyrazolidin-3-ones

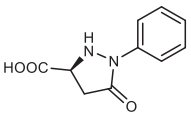


General procedure:

Step 1: The reaction mixture (20 mL) consisted of fumaric acid (**6**, 50 mM) and arylhydrazines (**9**, 10 mM) in degassed buffer (50 mM NaH_2PO_4-NaOH, pH 8.5) under argon atmosphere. The pH of the reaction mixture was adjusted to pH 8.5. The enzymatic reaction was started by addition of freshly purified EDDS lyase (0.1 mol%). The reaction mixture was then incubated at room temperature from 48 h to 96 h (Table 2). The progress of the enzymatic reaction was monitored by ^{1}H NMR spectroscopy by comparing signals of substrates and corresponding products. Without purification of the enzymatic product, the reaction mixture was subjected to the next step immediately.

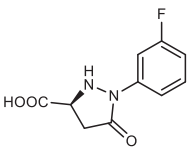
Step 2: 1.6 mL of fuming HCl (37 wt%) was added to the stirred reaction mixture from step 1 dropwise under ice-bath. After 5 min, the reaction mixture was heated to reflux for 3 h under nitrogen atmosphere. After completion of the reaction, the reaction mixture was allowed to cool down to room temperature. The reaction mixture was extracted with EtOAc (20 mL x 3), and the combined organic layers were washed with brine (30 mL). The organic solvent was evaporated to provide crude product, which was purified by C18 column chromatography (5% to 50% CH_3CN in H_2O as the eluent).

(*S*)-2-phenyl-5-carboxylpyrazolidin-3-one (one-pot **11a**)



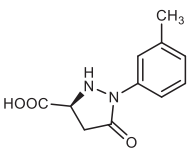
White solid. 28 mg (68% yield over two steps). ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.79 (d, J = 8.1 Hz, 2H), 7.37 – 7.34 (m, 2H), 7.14 (t, J = 7.4 Hz, 1H), 4.32 (dd, J = 8.8, 6.7 Hz, 1H), 3.08 (dd, J = 16.6, 8.7 Hz, 1H), 2.94 (dd, J = 16.6, 6.6 Hz, 1H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 174.0, 171.9, 139.8, 129.6 (2C), 125.9, 120.4 (2C), 56.4, 38.7. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}N_2O_3$ [M+H]$^+$: 207.0764, found: 207.0763. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R = 9.1 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S15).

(*S*)-2-(3-fluorophenyl)-5-carboxylpyrazolidin-3-one (one-pot **11c**)



White solid. 29 mg (65% yield over two steps). ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.68 – 7.63 (m, 2H), 7.37 – 7.32 (m, 1H), 6.86 (td, J = 8.5, 2.6 Hz, 1H), 4.32 (dd, J = 8.6, 6.8 Hz, 1H), 3.08 (dd, J = 16.7, 8.6 Hz, 1H), 2.94 (dd, J = 16.7, 6.8 Hz, 1H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 173.9, 172.4, 164.1 (d, J = 243.2 Hz), 141.5 (d, J = 10.1 Hz), 131.1 (d, J = 8.8 Hz), 115.3 (d, J = 18.9 Hz), 111.9 (d, J = 21.4 Hz), 106.8 (d, J = 26.5 Hz), 56.5, 38.8. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3F$ [M+H]$^+$: 225.0670, found: 225.0669. HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R = 14.9 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S17).

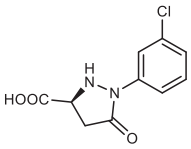
(*S*)-2-(3-methylphenyl)-5-carboxylpyrazolidin-3-one (one-pot **11d**)



White solid. 31 mg (70% yield over two steps). ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.62 (s, 1H), 7.58 (dd, J = 8.1, 2.1 Hz, 1H), 7.23 (t, J = 7.9 Hz, 1H), 6.98 (d, J = 7.5 Hz, 1H), 4.31 (dd, J = 8.8, 6.7 Hz, 1H), 3.07 (dd, J = 16.6, 8.8 Hz, 1H), 2.93 (dd, J = 16.6, 6.7 Hz, 1H), 2.34 (s, 3H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 174.0, 171.8, 139.7, 139.6,

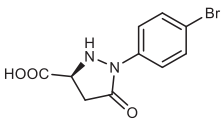
129.4, 126.6, 121.0, 117.7, 56.4, 38.7, 21.6. HRMS (ESI$^+$): calcd. for $C_{11}H_{13}N_2O_3$ $[M+H]^+$: 221.0921, found: 221.0918. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R = 11.8 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S18).

(*S*)-2-(3-chlorophenyl)-5-carboxylpyrazolidin-3-one (one-pot **11e**)



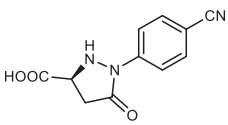
White solid. 33 mg (68% yield over two steps). ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.92 (t, J = 2.1 Hz, 1H), 7.78 – 7.75 (m, 1H), 7.33 (t, J = 8.2 Hz, 1H), 7.13 – 7.11 (m, 1H), 4.32 (dd, J = 8.6, 6.7 Hz, 1H), 3.09 (dd, J = 16.7, 8.6 Hz, 1H), 2.94 (dd, J = 16.7, 6.6 Hz, 1H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 173.8, 172.5, 141.2, 135.2, 130.9, 125.3, 119.7, 118.0, 56.5, 38.7. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3Cl$ $[M+H]^+$: 241.0374, found: 241.0373. Chiral HPLC conditions E: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 1.0 mL/min, rt, UV detection at 260 nm, t_R = 13.0 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S19).

(*S*)-2-(4-bromophenyl)-5-carboxylpyrazolidin-3-one (one-pot **11h**)



White solid. 35 mg (61% yield over two steps). ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.77 (d, J = 9.0 Hz, 2H), 7.49 (d, J = 8.9 Hz, 2H), 4.32 (dd, J = 8.6, 6.7 Hz, 1H), 3.07 (dd, J = 16.6, 8.6 Hz, 1H), 2.93 (dd, J = 16.6, 6.6 Hz, 1H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 173.9, 172.2, 139.2, 132.6, 132.5, 121.8, 121.6, 118.1, 56.4, 38.7. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3Br$ $[M+H]^+$: 284.9869, found: 284.9868. Chiral HPLC conditions F: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (25%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 1.0 mL/min, rt, UV detection at 260 nm, t_R = 14.3 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S22).

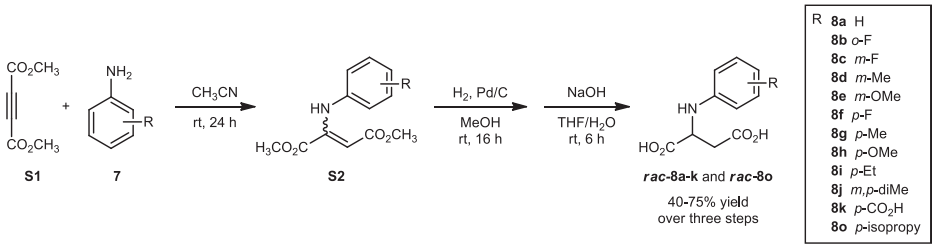
(*S*)-2-(4-cyanophenyl)-5-carboxylpyrazolidin-3-one (one-pot **11i**)



White solid. 29 mg (63% yield over two steps). ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.05 (d, J = 8.9 Hz, 2H), 7.70 (d, J = 9.0 Hz, 2H), 4.34 (dd, J = 8.5, 6.8 Hz, 1H), 3.10 (dd, J = 16.8, 8.5 Hz, 1H), 2.96 (dd, J = 16.8, 6.8 Hz, 1H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 173.7, 173.2, 143.9, 134.0, 133.8, 119.8, 119.5 (2C), 107.7, 56.5, 38.8. HRMS (ESI$^+$): calcd. for $C_{11}H_{10}N_3O_3$ $[M+H]^+$: 232.0717, found: 232.0716. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile

phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R = 18.9 min. The *ee* was determined to be >99% by chiral HPLC analysis using racemic standard (Figure S23).

7. Synthesis of *rac*-*N*-aryl-substituted Asp reference compounds

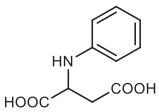


General procedure. To a stirred solution of dimethyl acetylenedicarboxylate (**S1**, 156 mg, 132 µL, 1.1 mmol) in CH_3CN (5 mL) was added the appropriate aromatic amine (**7**, 1.0 mmol). The reaction mixture was stirred at room temperature for 24 h. After completion of the reaction, the solvent was removed under reduced pressure to give the crude product (**S2**), which was directly used for the next step without purification.

To a stirred solution of **S2** in MeOH (5 mL) was added Pd/C (10.0 mg, 10 wt%) under nitrogen atmosphere. The reaction was stirred under H_2 atmosphere (balloon) for 16 h at room temperature. After completion of the reaction, the reaction mixture was filtered through Celite. The filtrate was concentrated *in vacuo* providing a crude oil which was directly used for the next step without purification.

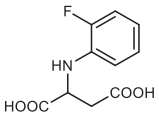
To a stirred solution of the crude oil in THF (2 mL) was added 2 M NaOH (2 mL), and the reaction mixture was stirred at room temperature for 6 h. After completion of the reaction, volatiles were removed *in vacuo*, and the residue was washed with EtOAc (2 mL). The aqueous layer was acidified with 1 M HCl (until pH=1) and loaded onto a column packed with cation-exchange resin (10 g of Dowex 50W X8, 50-100 mesh), which was pre-treated with 2 M aqueous ammonia (4 column volumes), 1 M HCl (2 column volumes) and distilled water (4 column volumes). The column was washed with distilled water (2 column volumes) and the product was eluted with 2 M aqueous ammonia (3 column volumes). The ninhydrin-positive fractions were collected and lyophilized to yield the desired product as ammonium salt.

Rac-N-phenylaspartic acid (*rac*-**8a**)



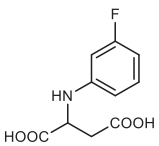
White solid. 130 mg (62% yield over three steps). ^{1}H NMR (500 MHz, D_2O): δ 7.25 – 7.22 (m, 2H), 6.82 (t, J = 7.3 Hz, 1H), 6.77 – 6.75 (m, 2H), 4.12 (dd, J = 9.8, 4.0 Hz, 1H), 2.69 (dd, J = 15.1, 4.0 Hz, 1H), 2.46 (dd, J = 15.1, 9.8 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.8, 179.0, 146.9, 129.5 (2C), 119.1, 114.7 (2C), 57.9, 40.5. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}NO_4$ $[M+H]^+$: 210.0761, found: 210.0761. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R (R) = 4.9 min, t_R (S) = 8.9 min (Figure S2).

Rac-N-(2-fluorophenyl)aspartic acid (*rac*-**8b**)



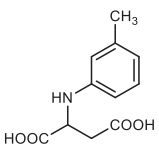
White solid. 91 mg (40% yield over three steps).^{1}H NMR (500 MHz, D_2O): δ 7.08 – 7.03 (m, 2H), 6.77 – 6.73 (m, 2H), 4.16 (dd, J = 9.9, 4.0 Hz, 1H), 2.76 (dd, J = 15.0, 4.0 Hz, 1H), 2.53 (dd, J = 15.0, 9.8 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.8, 178.9, 151.8 (d, J = 238.1 Hz), 135.7 (d, J = 11.3 Hz), 124.8, 118.2, 114.7 (d, J = 17.6 Hz), 113.8, 57.1, 40.6. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4F$ $[M+H]^+$: 228.0667, found: 228.0669.

Rac-N-(3-fluorophenyl)aspartic acid (*rac*-**8c**)



White solid. 135 mg (59% yield over three steps). ^{1}H NMR (500 MHz, D_2O): δ 7.17 (q, J = 7.6 Hz, 1H), 6.50 – 6.41 (m, 3H), 4.09 (dd, J = 10.1, 3.7 Hz, 1H), 2.72 (dd, J = 15.2, 3.7 Hz, 1H), 2.46 (dd, J = 15.1, 10.2 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 181.0, 178.9, 163.7 (d, J = 240.7 Hz), 149.6 (d, J = 11.3 Hz), 130.6, 109.7, 104.3, 100.2, 57.3, 40.6. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4F$ $[M+H]^+$: 228.0667, found: 228.0666. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4F$ $[M+H]^+$: 228.0667, found: 228.0664. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R (peak-1) = 4.7 min, t_R (peak-2) = 8.1 min (Figure S3).

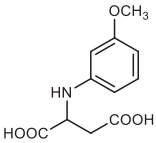
Rac-N-(3-methylphenyl)aspartic acid (*rac*-**8d**)



Light yellow solid. 95 mg (43% yield over three steps). ^{1}H NMR (500 MHz, D_2O): δ 7.14 (t, J = 7.8 Hz, 1H), 6.67 (d, J = 7.5 Hz, 1H), 6.61 (s, 1H), 6.57 (d, J = 8.2 Hz, 1H), 4.11 (dd, J = 10.1, 3.8 Hz, 1H), 2.69 (dd, J = 15.0, 3.9 Hz, 1H), 2.45 (dd, J = 15.0, 10.0 Hz, 1H), 2.25 (s, 3H); ^{13}C NMR (126 MHz, D_2O): δ 181.3, 179.3, 147.6, 139.8, 129.5, 119.4, 115.0, 111.6, 57.9, 41.0, 20.6. HRMS (ESI$^+$): calcd. for $C_{11}H_{14}NO_4$ $[M+H]^+$: 224.0917, found: 224.0918. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a

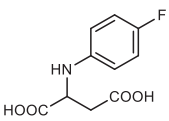
flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R (peak-1) = 5.9 min, t_R (peak-2) = 11.1 min (Figure S4).

Rac-N-(3-methoxyphenyl)aspartic acid (*rac*-**8e**)



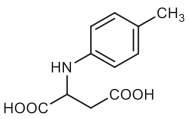
Light yellow solid. 173 mg (73% yield over three steps). ^{1}H NMR (500 MHz, D_2O): δ 7.17 (t, J = 8.0 Hz, 1H), 6.43 – 6.39 (m, 2H), 6.35 (s, 1H), 4.13 (dd, J = 9.9, 3.9 Hz, 1H), 3.78 (s, 3H), 2.72 (dd, J = 3.5, 15.2 Hz, 1H), 2.48 (dd, J = 15.1, 10.0 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 181.0, 179.1, 160.0, 149.2, 130.5, 107.5, 104.2, 99.8, 57.7, 55.3, 40.8. HRMS (ESI$^+$): calcd. for $C_{11}H_{14}NO_5$ $[M+H]^+$: 240.0866, found: 240.0867. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R (peak-1) = 6.1 min, t_R (peak-2) = 10.9 min (Figure S5).

Rac-N-(4-fluorophenyl)aspartic acid (*rac*-**8f**)



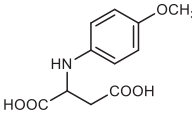
Light yellow solid. 170 mg (75% yield over three steps). ^{1}H NMR (500 MHz, D_2O): δ 7.00 (t, J = 8.8 Hz, 2H), 6.78 – 6.75 (m, 2H), 4.09 (dd, J = 9.8, 4.0 Hz, 1H), 2.69 (dd, J = 15.1, 4.0 Hz, 1H), 2.46 (dd, J = 15.1, 9.8 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.3, 178.8, 156.8 (d, J = 235.6 Hz), 142.6, 116.4 (d, J = 7.8 Hz, 2C), 115.7 (d, J = 22.7 Hz, 2C), 59.0, 40.2. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4F$ $[M+H]^+$: 228.0667, found: 228.0667. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R (*R*) = 4.4 min, t_R (*S*) = 6.7 min (Figure S6).

Rac-N-(4-methylphenyl)aspartic acid (*rac*-**8g**)



Light yellow solid. 153 mg (69% yield over three steps). ^{1}H NMR (500 MHz, D_2O): δ 7.17 (d, J = 8.1 Hz, 2H), 6.89 (d, J = 8.2 Hz, 2H), 4.12 (dd, J = 8.9, 4.1 Hz, 1H), 2.68 (dd, J = 15.8, 4.1 Hz, 1H), 2.55 (dd, J = 15.9, 8.9 Hz, 1H), 2.25 (s, 3H); ^{13}C NMR (126 MHz, D_2O): δ 178.7, 178.5, 141.0, 131.8, 130.0 (2C), 117.1 (2C), 59.2, 38.8, 19.6. HRMS (ESI$^+$): calcd. for $C_{11}H_{14}NO_4$ $[M+H]^+$: 224.0917, found: 224.0918. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R (*R*) = 5.5 min, t_R (*S*) = 10.8 min (Figure S7).

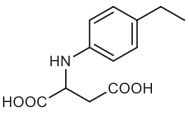
Rac-N-(4-methoxyphenyl)aspartic acid (*rac*-**8h**)



Light yellow solid. 135 mg (56% yield over three steps). ^{1}H NMR (500 MHz, 0.1 M NaOD/D_2O): δ 6.88 (d, J = 9.0 Hz, 2H), 6.73 (d, J = 9.0 Hz, 2H), 4.07 (dd, J = 10.1, 4.0 Hz, 1H), 3.76 (s, 3H), 2.67 (dd, J =

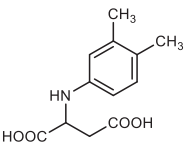
14.8, 4.0 Hz, 1H), 2.40 (dd, *J* = 14.8, 10.1 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 178.7, 178.6, 154.3, 137.2, 118.7 (2C), 115.1 (2C), 60.2, 55.8, 39.0. HRMS (ESI$^+$): calcd. for $C_{11}H_{14}NO_5$ $[M+H]^+$: 240.0866, found: 240.0868. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R (*R*) = 4.7 min, t_R (*S*) = 8.0 min (Figure S8).

Rac-N-(4-ethylphenyl)aspartic acid (*rac*-**8i**)



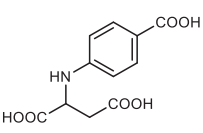
Light yellow solid. 140 mg (59% yield over three steps). ^{1}H NMR (500 MHz, D_2O): δ 7.18 (d, *J* = 8.4 Hz, 2H), 6.86 (d, *J* = 8.5 Hz, 2H), 4.11 (dd, *J* = 9.0, 4.1 Hz, 1H), 2.67 (dd, *J* = 15.7, 4.1 Hz, 1H), 2.57 – 2.49 (m, 3H), 1.14 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR (126 MHz, D_2O): δ 178.2, 178.00, 140.2, 139.4, 129.0 (2C), 117.8 (2C), 59.9, 38.2, 27.5, 15.0. HRMS (ESI$^+$): calcd. for $C_{12}H_{16}NO_4$ $[M+H]^+$: 238.1074, found: 238.1074. HRMS (ESI$^+$): calcd. for $C_{12}H_{16}NO_4$ $[M+H]^+$: 238.1074, found: 238.1073. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R (peak-1) = 8.3 min, t_R (peak-2) = 14.3 min (Figure S9).

Rac-N-(3,4-dimethylphenyl)aspartic acid (*rac*-**8j**)



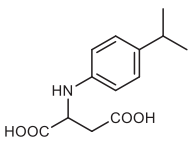
Light yellow solid. 129 mg (54% yield over three steps). ^{1}H NMR (500 MHz, D_2O): δ 7.15 (d, *J* = 8.1 Hz, 1H), 6.90 (d, *J* = 2.5 Hz, 1H), 6.82 (dd, *J* = 8.1, 2.6 Hz, 1H), 4.11 (dd, *J* = 8.1, 4.4 Hz, 1H), 2.66 (dd, *J* = 16.2, 4.4 Hz, 1H), 2.59 (dd, *J* = 16.2, 8.2 Hz, 1H), 2.22 (s, 3H), 2.19 (s, 3H); ^{13}C NMR (126 MHz, D_2O): δ 178.1, 177.5, 139.4, 138.5, 132.3, 130.4, 119.4, 115.5, 60.0, 37.8, 18.9, 18.0. HRMS (ESI$^+$): calcd. for $C_{12}H_{16}NO_4$ $[M+H]^+$: 238.1074, found: 238.1073. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R(peak-1) = 8.7 min, t_R(peak-2) = 15.1 min (Figure S10).

Rac-N-(4-carboxyphenyl)aspartic acid (*rac*-**8k**)



Light yellow solid. 130 mg (51% yield over three steps). ^{1}H NMR (500 MHz, D_2O): δ 7.75 (d, *J* = 8.7 Hz, 2H), 6.67 (d, *J* = 8.7 Hz, 2H), 4.18 (dd, *J* = 10.1, 3.8 Hz, 1H), 2.76 (dd, *J* = 15.2, 3.8 Hz, 1H), 2.51 (dd, *J* = 15.2, 10.2 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.7, 178.8, 174.5, 151.0, 131.2, 131.0, 122.6, 112.4, 112.2, 56.7, 40.4. HRMS (ESI$^+$): calcd. for $C_{11}H_{12}NO_6$ $[M+H]^+$: 254.0659, found: 254.0658. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R (peak-1) = 3.8 min, t_R (peak-2) = 4.5 min (Figure S11).

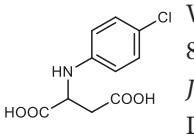
Rac-N-(4-isopropylphenyl)aspartic acid (*rac*-**8o**)



Light yellow solid. 150 mg (60% yield over three steps). ^{1}H NMR (500 MHz, 0.1 M NaOD/D_2O): δ 7.16 (d, J = 8.5 Hz, 2H), 6.70 (d, J = 8.5 Hz, 2H), 4.10 (dd, J = 10.1, 3.9 Hz, 1H), 2.81 (hept, J = 6.2 Hz, 1H), 2.68 (dd, J = 14.8, 3.8 Hz, 1H), 2.41 (dd, J = 14.8, 10.2 Hz, 1H), 1.16 (d, J = 6.9 Hz, 6H); ^{13}C NMR (126 MHz, 0.1 M NaOD/D_2O): δ 181.7, 179.4, 145.8, 139.4, 127.2 (2C), 114.6, 114.4, 58.0, 41.2, 32.6, 23.4 (2C). HRMS (ESI$^+$): calcd. for $C_{13}H_{18}NO_4$ $[M+H]^+$: 252.1230, found: 252.1230.

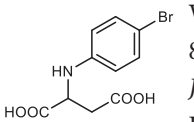
General procedure. To a stirred solution of *rac*-2-bromosuccinic acid (**S3**, 392 mg, 2.0 mmol) in H_2O/THF (5 mL, 9:1, v/v) was added the appropriate aromatic amine (**7**, 0.4 mmol). The pH of the reaction mixture was adjusted to pH 7.5 using 2 M NaOH aqueous solution. The reaction mixture was heated to reflux for 1 h. After completion of the reaction, volatiles were removed *in vacuo*, and the residue was washed with EtOAc (5 mL). The aqueous layer was acidified with 1 M HCl (until pH=1) and loaded onto a column packed with cation-exchange resin (10 g of Dowex 50W X8, 50-100 mesh), which was pre-treated with 2 M aqueous ammonia (4 column volumes), 1 M HCl (2 column volumes) and distilled water (4 column volumes). The column was washed with distilled water (2 column volumes) and the product was eluted with 2 M aqueous ammonia (2 column volumes). The ninhydrin-positive fractions were collected and lyophilized to yield the desired product as ammonium salt.

Rac-N-(4-chlorophenyl)aspartic acid (*rac*-**8l**)



White solid. 61 mg (62% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.21 (d, J = 8.8 Hz, 2H), 6.70 (d, J = 8.8 Hz, 2H), 4.11 (dd, J = 9.8, 4.0 Hz, 1H), 2.73 (dd, J = 15.3, 4.0 Hz, 1H), 2.49 (dd, J = 15.2, 9.8 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.6, 178.7, 146.0, 129.0 (2C), 122.6, 115.6, 115.4, 57.5, 40.3. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4Cl$ $[M+H]^+$: 244.0371, found: 244.0370. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R (peak-1) = 4.6 min, t_R (peak-2) = 8.4 min (Figure S12).

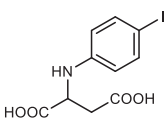
Rac-N-(4-bromophenyl)aspartic acid (*rac*-**8m**)



White solid. 50 mg (43% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.34 (d, J = 8.8 Hz, 2H), 6.65 (d, J = 8.8 Hz, 2H), 4.10 (dd, J = 9.8, 4.0 Hz, 1H), 2.73 (dd, J = 15.3, 4.1 Hz, 1H), 2.49 (dd, J = 15.3, 9.8 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.4, 178.5, 146.4, 131.9 (2C), 115.5 (2C), 109.6, 57.0, 40.2. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4Br$ $[M+H]^+$: 287.9866, found: 287.9864. Chiral HPLC

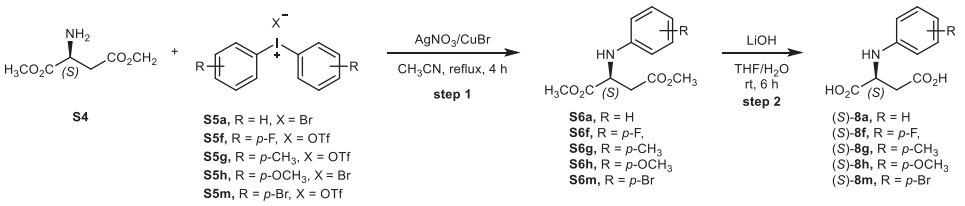
condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R (*R*) = 5.4 min, t_R (*S*) = 9.7 min (Figure S13).

Rac-N-(4-iodophenyl)aspartic acid (*rac*-**8n**)



White solid. 34 mg (25% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.53 (d, J = 8.7 Hz, 2H), 6.56 (d, J = 8.4 Hz, 2H), 4.12 (dd, J = 9.5, 4.2 Hz, 1H), 2.75 (dd, J = 15.4, 4.2 Hz, 1H), 2.53 (dd, J = 15.4, 9.5 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.1, 178.1, 146.8, 137.9, 137.8, 116.6, 116.4, 79.07, 57.0, 39.7. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4I$ $[M+H]^+$: 335.9727, found: 335.9727. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R (peak-1) = 7.5 min, t_R (peak-2) = 15.5 min (Figure S14).

8. Synthesis of chiral (S)-*N*-aryl-substituted Asp reference compounds



S4

S5a, R = H, X = Br
S5f, R = *p*-F, X = OTf
S5g, R = *p*-CH_3, X = OTf
S5h, R = *p*-OCH_3, X = Br
S5m, R = *p*-Br, X = OTf

S6a, R = H
S6f, R = *p*-F,
S6g, R = *p*-CH_3
S6h, R = *p*-OCH_3
S6m, R = *p*-Br

(*S*)-**8a,** R = H
(*S*)-**8f,** R = *p*-F,
(*S*)-**8g,** R = *p*-CH_3
(*S*)-**8h,** R = *p*-OCH_3
(*S*)-**8m,** R = *p*-Br

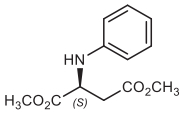
General procedure:

Step 1. The synthetic procedure was according to a procedure published elsewhere.[2] To a stirred solution of dimethyl (*S*)-aspartate (**S4**, 80 mg, 0.50 mmol, free amine) in dry CH_3CN (5 mL) was added the appropriate diaryliodonium salt (**S5**, 0.25 mmol), $AgNO_3$ (44 mg, 0.26 mmol) and CuBr (1 mg). The system was protected from light, flushed with N_2 and heated to reflux for 3 h. After completion of the reaction, Na_2CO_3 (100 mg) was added, and the reaction mixture was filtered. The filtrate was removed under reduced pressure to give the crude product (**S6**) which was purified via flash chromatography (EtOAc/Petroleum ether 15%, v/v) to give **S6** as pure product.

Step 2. To a stirred solution of dimethyl (*S*)-*N*-substituted aspartate **S6** (**S6a**, 27 mg, 0.11 mmol; **S6f**, 25 mg, 0.10 mmol; **S6g**, 30 mg, 0.12 mmol; **S6h**, 30 mg, 0.11 mmol; **S3m**, 35 mg, 0.11 mmol; respectively) in THF/H_2O (1:1, each 1 mL) was added LiOH (4 e.q.), and the reaction mixture was stirred at room temperature for 6 h. Volatiles were removed

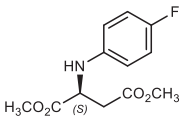
in vacuo, and the residue was washed with EtOAc (1 mL). The aqueous layer was acidified with 1 M HCl (until pH=1) and loaded onto a column packed with cation-exchange resin (5 g of Dowex 50W X8, 50-100 mesh), which was pre-treated with 2 M aqueous ammonia (4 column volumes), 1 M HCl (2 column volumes) and distilled water (4 column volumes). The column was washed with distilled water (2 column volumes) and the product was eluted with 2 M aqueous ammonia (2 column volumes). The ninhydrin-positive fractions were collected and lyophilized to yield the desired product as ammonium salt.

Dimethyl (*S*)-*N*-phenyl-aspartate (**S6a**)



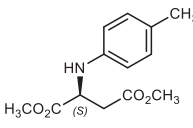
Clear oil. 31 mg (52% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.19 (dd, J = 8.5, 7.4 Hz, 2H), 6.77 (t, J = 7.3 Hz, 1H), 6.67 (d, J = 7.7 Hz, 2H), 4.47 – 4.44 (m, 2H), 3.76 (s, 3H), 3.70 (s, 3H), 2.89 – 2.88 (m, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.9, 171.1, 146.3, 129.5 (2C), 118.9, 113.8 (2C), 53.5, 52.7, 52.1, 37.3. HRMS (ESI$^+$): calcd. for $C_{12}H_{16}NO_4$ $[M+H]^+$: 238.1074, found: 238.1072.

Dimethyl (*S*)-*N*-(4-fluorophenyl)aspartate (**S6f**)



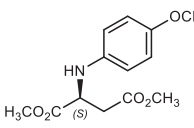
Clear oil. 25 mg (39% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.89 (t, J = 8.7 Hz, 2H), 6.63 – 6.60 (m, 2H), 4.39 – 4.32 (m, 2H), 3.74 (s, 3H), 3.70 (s, 3H), 2.85 (d, J = 5.5 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.8, 171.0, 156.6 (d, J = 238.1 Hz), 142.6 (d, J = 1.3 Hz), 115.8 (d, J = 22.6 Hz, 2C), 115.1 (d, J = 7.6 Hz, 2C), 54.3, 52.6, 52.1, 37.2. HRMS (ESI$^+$): calcd. for $C_{12}H_{15}NO_4F$ $[M+H]^+$: 256.098, found: 256.0971.

Dimethyl (*S*)-*N*-(4-methylphenyl)aspartate (**S6g**)



Clear oil. 40 mg (64% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.00 (d, J = 8.0 Hz, 2H), 6.59 (d, J = 7.8 Hz, 2H), 4.43 (d, J = 5.7 Hz, 1H), 4.32 (d, J = 6.8 Hz, 1H), 3.74 (s, 3H), 3.69 (s, 3H), 2.87 (d, J = 5.7 Hz, 2H), 2.24 (s, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.1, 171.1, 144.0, 130.0 (2C), 128.2, 114.2 (2C), 54.0, 52.6, 52.1, 37.3, 20.5. HRMS (ESI$^+$): calcd. for $C_{13}H_{18}NO_4$ $[M+H]^+$: 252.1230, found: 252.1229.

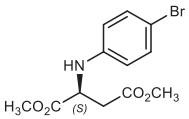
Dimethyl (*S*)-*N*-(4-methoxyphenyl)aspartate (**S6h**)



Clear oil. 50 mg (75% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.78 (d, J = 8.9 Hz, 2H), 6.65 (d, J = 8.9 Hz, 2H), 4.37 (t, J = 5.9 Hz, 1H), 4.16 (s, 1H), 3.74 (s, 6H), 3.70 (s, 3H), 2.85 (d, J = 5.9 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 173.1, 171.1, 153.2, 140.3, 115.8 (2C), 114.9 (2C),

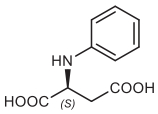
55.7, 54.9, 52.5, 52.0, 37.4. HRMS (ESI$^+$): calcd. for $C_{13}H_{18}NO_5$ [M+H]$^+$: 268.1179, found: 268.1174.

Dimethyl (*S*)-*N*-(4-bromophenyl)aspartate (**S6m**)



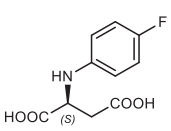
Clear oil. 48 mg (61% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.28 (d, J = 8.7 Hz, 2H), 6.56 (d, J = 8.8 Hz, 2H), 4.53 (d, J = 9.1 Hz, 1H), 4.44 – 4.40 (m, 1H), 3.77 (s, 3H), 3.72 (s, 3H), 2.89 (dd, J = 5.6, 1.9 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 172.6, 171.0, 145.4, 132.2 (2C), 115.5 (2C), 110.7, 53.5, 52.8, 52.2, 37.1. HRMS (ESI$^+$): calcd. for $C_{12}H_{15}NO_4Br$ [M+H]$^+$: 316.0179, found: 316.0178.

(*S*)-*N*-phenylaspartic acid [(*S*)-**8a**]



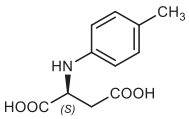
White solid. 19 mg (80% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.27 (t, J = 7.7 Hz, 2H), 6.88 (t, J = 7.4 Hz, 1H), 6.82 (d, J = 8.0 Hz, 2H), 4.14 (dd, J = 9.6, 4.0 Hz, 1H), 2.71 (dd, J = 15.3, 4.0 Hz, 1H), 2.50 (dd, J = 15.3, 9.6 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.3, 178.7, 146.2, 129.5 (2C), 119.6, 115.1 (2C), 58.4, 40.0. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}NO_4$ [M+H]$^+$: 210.0761, found: 210.0760. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R (*R*) = 5.1 min, t_R (*S*) = 8.6 min. The *ee* was determined to be 94.1% by chiral HPLC analysis using racemic standard (Figure S2).

(*S*)-*N*-(4-fluorophenyl)aspartic acid [(*S*)-**8f**]



White solid. 15 mg (67% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.02 (t, J = 8.9 Hz, 2H), 6.84 – 6.82 (m, 2H), 4.10 (dd, J = 9.4, 4.1 Hz, 1H), 2.70 (dd, J = 15.5, 4.1 Hz, 1H), 2.51 (dd, J = 15.5, 9.4 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 179.9, 178.6, 157.1 (d, J = 235.6 Hz), 141.93, 116.86 (d, J = 11.3 Hz, 2C), 115.8 (d, J = 22.6 Hz, 2C), 58.6, 39.8. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4F$ [M+H]$^+$: 228.0667, found: 228.0661. Chiral HPLC condition A: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm, t_R (*R*) = 4.6 min, t_R (*S*) = 7.0 min. The *ee* was determined to be 97.2% by chiral HPLC analysis using racemic standard (Figure S6).

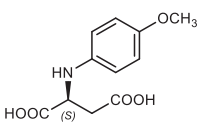
(*S*)-*N*-(4-methylphenyl)aspartic acid [(*S*)-**8g**]



White solid. 15 mg (56% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.18 (d, J = 8.0 Hz, 2H), 6.93 (d, J = 7.9 Hz, 2H), 4.13 (dd, J = 8.7, 4.2 Hz, 1H), 2.68 (dd, J = 16.1, 4.2 Hz, 1H), 2.58 (dd, J = 16.1, 8.5 Hz, 1H), 2.26 (s, 3H); ^{13}C NMR (126 MHz, D_2O): δ 178.9, 178.5, 141.3, 131.8, 130.0 (2C),

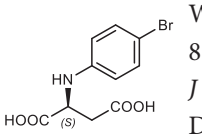
117.0 (2C), 59.2, 39.0, 19.6. HRMS (ESI$^+$): calcd. for $C_{11}H_{14}NO_4$ [M+H]$^+$: 224.0917, found: 224.0916. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R (*R*) = 6.3 min, t_R (*S*) = 10.2 min. The *ee* was determined to be 98.7% by chiral HPLC analysis using racemic standard (Figure S7).

(*S*)-*N*-(4-methoxyphenyl)aspartic acid [(*S*)-**8h**]



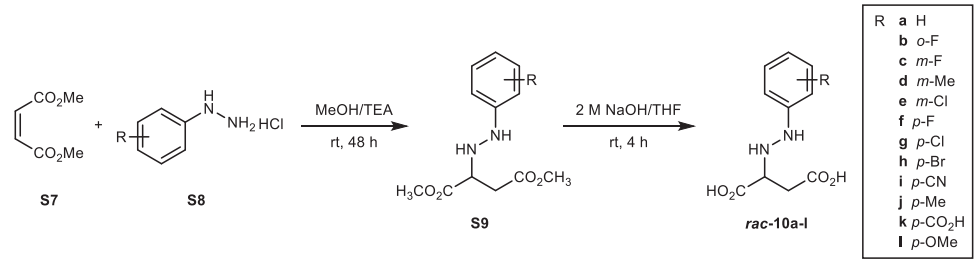
White solid. 23 mg (85% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.04 (d, *J* = 9.0 Hz, 2H), 6.97 (d, *J* = 9.1 Hz, 2H), 4.10 (dd, *J* = 8.6, 4.1 Hz, 1H), 3.79 (s, 3H), 2.66 (dd, *J* = 16.1, 4.1 Hz, 1H), 2.57 (dd, *J* = 16.1, 8.6 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 178.3, 177.7, 155.2, 135.4, 119.7 (2C), 115.2 (2C), 60.8, 55.8, 38.1. HRMS (ESI$^+$): calcd. for $C_{11}H_{14}NO_5$ [M+H]$^+$: 240.0866, found: 240.0858. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R (*R*) = 5.1 min, t_R (*S*) = 8.0 min. The *ee* was determined to be 98.7% by chiral HPLC analysis using racemic standard (Figure S8).

(*S*)-*N*-(4-bromophenyl)aspartic acid [(*S*)-**8m**]



White solid. 19 mg (59% yield). ^{1}H NMR (500 MHz, D_2O): δ 7.34 (d, *J* = 8.9 Hz, 2H), 6.66 (d, *J* = 8.9 Hz, 2H), 4.11 (dd, *J* = 9.6, 4.1 Hz, 1H), 2.74 (dd, *J* = 15.3, 4.1 Hz, 1H), 2.51 (dd, *J* = 15.3, 9.6 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.3, 178.4, 146.4, 131.9 (2C), 116.1 (2C), 109.7, 57.0, 40.0. HRMS (ESI$^+$): calcd. for $C_{10}H_{11}NO_4Br$ [M+H]$^+$: 287.9866, found: 287.9866. Chiral HPLC condition B: Nucleosil chiral-1 column with 0.5 mM aqueous $CuSO_4$ as mobile phase with a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm, t_R (*R*) = 5.6 min, t_R (*S*) = 9.5 min. The *ee* was determined to be 98.7% by chiral HPLC analysis using racemic standard (Figure S13).

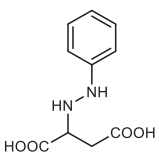
9. Synthesis of *rac-N*-(phenylamino)aspartic acid analogues



General procedure. To a stirred solution of the appropriate arylhydrazine hydrochloride (**S8**, 2.5 mmol) in dry CH_3OH (10 mL) was added triethylamine (260 mg, 360 µL, 2.75 mmol) dropwise under nitrogen atmosphere. The dimethyl maleate (**S7**, 356 mg, 310 µL, 3.12 mmol) was added to the reaction mixture. The reaction mixture was stirred at room temperature for 48 h. After completion of the reaction, the solvent was removed under reduced pressure. The resulting oil was dissolved in 1 M HCl (10 mL) and extracted with EtOAc (10 mL x 3), washed with brine (30 mL) and dried over Na_2SO_4. The solvent was evaporated to provide crude product (**S9**), which was directly used for the next step without purification.

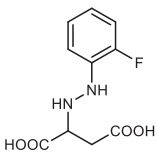
To a stirred solution of crude **S9** in THF (3 mL) was added 2 M NaOH (3 mL) under nitrogen atmosphere. The reaction was stirred 4 h at room temperature. After completion of the reaction, volatiles were removed *in vacuo*, and the residue was washed with EtOAc (5 mL x 3). The aqueous layer was acidified with 1 M HCl (until pH=1) and loaded onto a column packed with cation-exchange resin (15 g of Dowex 50W X8, 50-100 mesh), which was pre-treated with 2 M aqueous ammonia (4 column volumes), 1 M HCl (2 column volumes) and distilled water (4 column volumes). The column was washed with distilled water (2 column volumes) and the product was eluted with 2 M aqueous ammonia (2 column volumes). The ninhydrin-positive fractions were collected and lyophilized to yield the desired product as ammonium salt.

Rac-N-(phenylamino)aspartic acid (*rac*-**10a**)



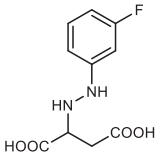
White solid. 313 mg (two-step yield 56%). ^{1}H NMR (500 MHz, D_2O): δ 7.33 – 7.30 (m, 2H), 7.06 – 7.03 (m, 2H), 6.99 – 6.96 (m, 1H), 3.78 (dd, J = 9.7, 4.0 Hz, 1H), 2.66 (dd, J = 15.7, 4.0 Hz, 1H), 2.42 (dd, J = 15.7, 9.7 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 178.9, 178.8, 146.7, 129.5 (2C), 121.4, 115.5 (2C), 61.1, 38.4. HRMS (ESI$^+$): calcd. for $C_{10}H_{13}N_2O_4$ $[M+H]^+$: 225.0870, found: 225.0867.

Rac-N-[(2-fluorophenyl)amino]aspartic acid (*rac*-**10b**)



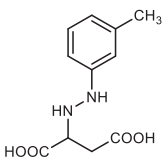
Light yellow solid. 320 mg (two-step yield 53%). ^{1}H NMR (500 MHz, D_2O): δ 7.30 (t, J = 8.3 Hz, 1H), 7.15 – 7.06 (m, 2H), 6.93 – 6.89 (m, 1H), 3.74 (dd, J = 9.7, 4.0 Hz, 1H), 2.64 (dd, J = 15.6, 4.1 Hz, 1H), 2.41 (dd, J = 15.6, 9.7 Hz, 1H); ^{13}C NMR (126 MHz, D_2O) δ: 179.4, 179.0, 152.0 (d, J = 239.4 Hz), 134.8 (d, J = 10.1 Hz), 124.77, 121.13, 117.01, 115.0 (d, J = 18.9 Hz), 61.3, 38.7. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}N_2O_4F$ $[M+H]^+$: 243.0776, found: 243.0773.

Rac-N-[(3-fluorophenyl)amino]aspartic acid (*rac*-**10c**)



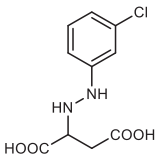
Light yellow solid. 321 mg (two-step yield 53%). ^{1}H NMR (500 MHz, D_2O): δ 7.25 – 7.21 (m, 1H), 6.83 – 6.75 (m, 2H), 6.60 (td, J = 8.7, 2.5 Hz, 1H), 3.73 (dd, J = 10.0, 4.0 Hz, 1H), 2.63 (dd, J = 15.5, 3.9 Hz, 1H), 2.37 (dd, J = 15.5, 10.0 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 179.8, 179.2, 163.6 (d, J = 241.9 Hz), 149.9 (d, J = 10.1 Hz), 130.5 (d, J = 10.1 Hz), 110.0 (d, J = 12.6 Hz), 106.4, 101.2 (d, J = 25.2 Hz), 61.6, 39.0. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}N_2O_4F$ $[M+H]^+$: 243.0776, found: 243.0776.

Rac-N-[(3-methylphenyl)amino]aspartic acid (*rac*-**10d**)



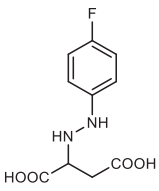
Light yellow solid. 500 mg (two-step yield 84%). ^{1}H NMR (500 MHz, D_2O): δ 7.20 (t, J = 7.8 Hz, 1H), 6.89 (s, 1H), 6.85 – 6.81 (m, 2H), 3.77 (dd, J = 9.5, 4.1 Hz, 1H), 2.66 (dd, J = 15.8, 4.1 Hz, 1H), 2.43 (dd, J = 15.8, 9.5 Hz, 1H), 2.28 (s, 3H); ^{13}C NMR (126 MHz, D_2O): δ 178.8, 178.6, 146.5, 139.8, 129.4, 122.1, 116.1, 112.6, 61.0, 38.2, 20.5. HRMS (ESI$^+$): calcd. for $C_{11}H_{15}N_2O_4$ $[M+H]^+$: 239.1026, found: 239.1023.

Rac-N-[(3-chlorophenyl)amino]aspartic acid (*rac*-**10e**)



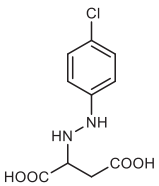
Light yellow solid. 341 mg (two-step yield 53%). ^{1}H NMR (500 MHz, D_2O): δ 7.20 (t, J = 8.1 Hz, 1H), 7.07 (t, J = 2.2 Hz, 1H), 6.89 – 6.86 (m, 2H), 3.71 (dd, J = 9.7, 4.1 Hz, 1H), 2.63 (dd, J = 15.5, 4.1 Hz, 1H), 2.37 (dd, J = 15.5, 9.8 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 179.7, 179.0, 149.1, 134.3, 130.4, 119.8, 114.1, 112.7, 61.4, 38.8. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}N_2O_4Cl$ $[M+H]^+$: 259.0480, found: 259.0479.

Rac-N-[(4-fluorophenyl)amino]aspartic acid (*rac*-**10f**)



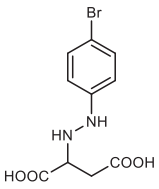
Light yellow solid. 357 mg (two-step yield 59%). ^{1}H NMR (500 MHz, D_2O): δ 7.04 – 7.03 (m, 4H), 3.75 (dd, J = 9.7, 4.0 Hz, 1H), 2.65 (dd, J = 15.8, 4.0 Hz, 1H), 2.41 (dd, J = 15.8, 9.7 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 178.9, 178.8, 157.9 (d, J = 235.6 Hz), 142.5, 117.5 (2C), 115.7 (d, J = 18.9 Hz, 2C), 61.0, 38.4. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}N_2O_4F$ $[M+H]^+$: 243.0776, found: 243.0775.

Rac-N-[(4-chlorophenyl)amino]aspartic acid (*rac*-**10g**)



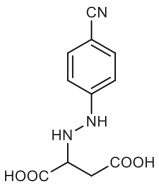
Light yellow solid. 387 mg (two-step yield 60%). ^{1}H NMR (500 MHz, D_2O): δ 7.30 (d, J = 8.8 Hz, 2H), 7.02 (d, J = 8.9 Hz, 2H), 3.76 (dd, J = 9.8, 4.0 Hz, 1H), 2.67 (dd, J = 15.5, 4.0 Hz, 1H), 2.42 (dd, J = 15.5, 9.8 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 179.5, 179.1, 146.1, 129.0 (2C), 124.7, 116.3 (2C), 61.4, 38.8. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}N_2O_4Cl$ $[M+H]^+$: 259.0480, found: 259.0482.

Rac-N-[(4-bromophenyl)amino]aspartic acid (*rac*-**10h**)



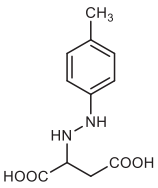
Light yellow solid. 356 mg (two-step yield 47%). ^{1}H NMR (500 MHz, D_2O): δ 7.43 (d, J = 8.8 Hz, 2H), 6.97 (d, J = 8.8 Hz, 2H), 3.76 (dd, J = 9.8, 4.0 Hz, 1H), 2.67 (dd, J = 15.5, 4.1 Hz, 1H), 2.42 (dd, J = 15.5, 9.8 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 179.6, 179.1, 146.7, 131.9 (2C), 116.6 (2C), 111.8, 61.4, 38.8. HRMS (ESI$^+$): calcd. for $C_{10}H_{12}N_2O_4Br$ $[M+H]^+$: 302.9975, found: 302.9977.

Rac-N-[(4-cyanophenyl)amino]aspartic acid (*rac*-**10i**)



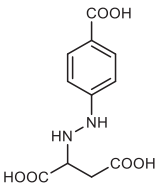
Yellow solid. 180 mg (two-step yield 29%). ^{1}H NMR (500 MHz, D_2O): δ 7.54 (d, J = 8.8 Hz, 2H), 6.98 (d, J = 8.9 Hz, 2H), 3.69 (dd, J = 10.2, 3.9 Hz, 1H), 2.62 (dd, J = 15.3, 3.9 Hz, 1H), 2.35 (dd, J = 15.3, 10.3 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 180.3, 179.3, 152.8, 133.9, 133.8, 121.5 (2C), 112.5, 98.4, 62.2, 39.0. HRMS (ESI$^+$): calcd. for $C_{11}H_{12}N_3O_4$ $[M+H]^+$: 250.0822, found: 250.0824.

Rac-N-[(4-methylphenyl)amino]aspartic acid (*rac*-**10j**)



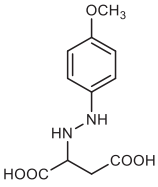
Light yellow solid. 375 mg (two-step yield 63%). ^{1}H NMR (500 MHz, D_2O): δ 7.16 (d, J = 8.2 Hz, 2H), 6.97 (d, J = 8.1 Hz, 2H), 3.76 (dd, J = 9.5, 4.1 Hz, 1H), 2.65 (dd, J = 15.8, 4.1 Hz, 1H), 2.43 (dd, J = 15.8, 9.5 Hz, 1H), 2.25 (s, 3H); ^{13}C NMR (126 MHz, D_2O): δ 178.9, 178.5, 143.5, 131.8, 129.8 (2C), 116.4 (2C), 60.8, 38.2, 19.6. HRMS (ESI$^+$): calcd. for $C_{11}H_{15}N_2O_4$ $[M+H]^+$: 239.1026, found: 239.1027.

Rac-N-[(4-carboxyphenyl)amino]aspartic acid (*rac*-**10k**)



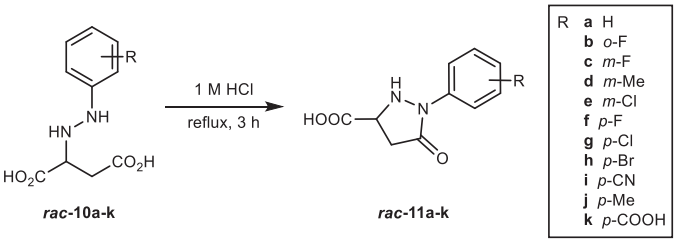
Yellow solid. 523 mg (two-step yield 65%). ^{1}H NMR (500 MHz, D_2O): δ 7.76 (d, J = 8.8 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 3.74 (dd, J = 9.8, 4.1 Hz, 1H), 2.63 (dd, J = 15.4, 4.1 Hz, 1H), 2.39 (dd, J = 15.4, 9.8 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 179.9, 179.2, 175.3, 150.9, 130.8, 130.6, 126.3, 112.9, 112.8, 61.8, 38.9. HRMS (ESI$^+$): calcd. for $C_{11}H_{13}N_2O_6$ $[M+H]^+$: 269.0768, found: 269.0766.

Rac-N-[(4-methoxyphenyl)amino]aspartic acid (*rac*-**10l**)



Orange solid. 222 mg (two-step yield 35%). ^{1}H NMR (500 MHz, D_2O): δ 7.09 – 7.07 (m, 2H), 6.97 – 6.96 (m, 2H), 3.78 – 3.81 (m, 4H), 2.69 (dd, J = 16.1, 4.1 Hz, 1H), 2.48 (dd, J = 16.1, 9.4 Hz, 1H); ^{13}C NMR (126 MHz, D_2O): δ 178.7, 177.8, 154.6, 139.1, 119.0 (2C), 114.9 (2C), 60.6, 55.7, 37.8. HRMS (ESI$^+$): calcd. for $C_{11}H_{14}N_2O_5$ $[M+H]^+$: 254.0897, found: 254.0895.

10. Synthesis of *rac*-2-aryl-5-carboxylpyrazolidin-3-ones (*rac*-11a-k)



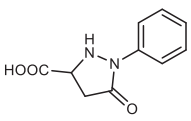
rac-**10a-k** *rac*-**11a-k**

General procedure. The *rac-N*-(arylamino)aspartic acid (*rac*-**10a**, 300 mg, 1.34 mmol; *rac*-**10b**, 280 mg, 1.16 mmol; *rac*-**10c**, 291 mg, 1.20 mmol; *rac*-**10d**, 189 mg, 0.79 mmol; *rac*-**10e**, 322 mg, 1.25 mmol; *rac*-**10f**, 130 mg, 0.54 mmol; *rac*-**10g**, 350 mg, 1.35 mmol; *rac*-**10h**, 343 mg, 1.13 mmol; *rac*-**10i**, 200 mg, 0.80 mmol; *rac*-**10j**, 450 mg, 1.89 mmol; *rac*-**10k**,

154 mg, 0.57 mmol) was dissolved in 1 M HCl aqueous solution (5 mL). The reaction mixture was heated to reflux for 3 h under nitrogen atmosphere. After completion of the reaction, the reaction mixture was allowed to cool down to room temperature and then kept in an ice-bath for 30 min.

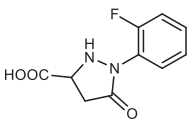
For compound *rac*-**11a**, *rac*-**11c-e**, *rac*-**11g-h** and *rac*-**11j-k**, the desired product was precipitated from the reaction mixture. The desired product was filtered off, washed with cold water (3 mL) and dried under vacuum overnight. For compound *rac*-**11f** and *rac*-**11i**, the reaction mixture was extracted with EtOAc (5 mL x 3). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4 and evaporated to provide pure product. For compound *rac*-**11b**, the reaction mixture was extracted with EtOAc (5 mL x 3). The combined organic layers were washed with brine (10 mL), dried over Na_2SO_4 and evaporated to provide crude product, which was further purified via C18 column chromatography (5% to 50% CH_3CN in H_2O as the eluent).

Rac-2-phenyl-5-carboxylpyrazolidin-3-one (*rac*-**11a**)



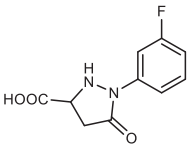
Light yellow solid. 180 mg (65% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.98 (brs, 1H), 7.78 – 7.76 (m, 2H), 7.35 (dd, J = 8.7, 7.3 Hz, 2H), 7.09 (tt, J = 7.3, 1.2 Hz, 1H), 6.49 (brs, 1H), 4.24 (dd, J = 8.6, 5.8 Hz, 1H), 2.98 (dd, J = 16.4, 8.6 Hz, 1H), 2.76 (dd, J = 16.4, 5.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.3, 170.1, 139.0, 128.5 (2C), 123.6, 118.0, 117.8, 54.8, 37.3. The NMR data are in agreement with published data.[1] HRMS (ESI$^+$): calcd. for $C_{10}H_{11}N_2O_3$ $[M+H]^+$: 207.0764, found: 207.0762. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R (*S*) = 9.1 min, t_R (*R*) = 11.2 min (Figure S15).

Rac-2-(2-fluorophenyl)-5-carboxylpyrazolidin-3-one (*rac*-**11b**)



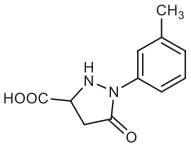
White solid. 95 mg (35% yield). ^{1}H NMR (500 MHz, Methanol-d_4): δ 7.53 (td, J = 7.9, 1.8 Hz, 1H), 7.38 – 7.34 (m, 1H), 7.24 – 7.19 (m, 2H), 4.38 (dd, J = 8.9, 5.9 Hz, 1H), 3.09 (dd, J = 16.7, 8.9 Hz, 1H), 2.91 (dd, J = 16.6, 5.9 Hz, 1H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 172.6, 171.3, 156.8 (d, J = 252.0 Hz), 129.2 (d, J = 8.8 Hz), 127.4, 125.1 (d, J = 12.6 Hz), 124.2 (d, J = 3.8 Hz), 116.0 (d, J = 20.2 Hz), 56.3, 35.6. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3F$ $[M+H]^+$: 225.0670, found: 225.0667. Chiral HPLC conditions D: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (15%, v/v, 0.1% TFA) as mobile phase with a flow rate of 0.25 mL/min, rt, UV detection at 260 nm, t_R (peak-1) = 14.0 min, t_R (peak-2) = 16.0 min (Figure S16).

Rac-2-(3-fluorophenyl)-5-carboxylpyrazolidin-3-one (*rac*-**11c**)



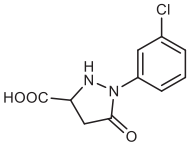
Light yellow solid. 187 mg (69% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.03 (brs, 1H), 7.63 – 7.60 (m, 2H), 7.42 – 7.37 (m, 1H), 6.92 (td, J = 8.4, 2.4 Hz, 1H), 6.60 (brs, 1H), 4.26 (dd, J = 8.5, 5.8 Hz, 1H), 3.01 (dd, J = 16.5, 8.6 Hz, 1H), 2.78 (dd, J = 16.6, 5.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.2, 170.7, 162.0 (d, J = 241.9 Hz), 140.5 (d, J = 11.3 Hz), 130.3 (d, J = 8.8 Hz), 113.5 (d, J = 13.9 Hz), 110.0 (d, J = 23.9 Hz), 104.4 (d, J = 40.3 Hz), 54.8, 37.4. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3F$ [M+H]$^+$: 225.0670, found: 225.0667. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R (*S*) = 14.5 min, t_R (*R*) = 17.8 min (Figure S17).

Rac-2-(3-methylphenyl)-5-carboxylpyrazolidin-3-one (*rac*-**11d**)



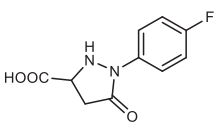
Light yellow solid. 130 mg (75% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.96 (brs, 1H), 7.61 – 7.59 (m, 2H), 7.22 (t, J = 7.8 Hz, 1H), 6.91 (d, J = 7.5 Hz, 1H), 6.52 (brs, 1H), 4.22 (dd, J = 8.6, 5.8 Hz, 1H), 2.97 (dd, J = 16.4, 8.6 Hz, 1H), 2.75 (dd, J = 16.4, 5.8 Hz, 1H), 2.30 (s, 3H); ^{13}C NMR (126 MHz, DMSO): δ 172.3, 167.0, 139.0, 137.7, 128.3, 124.3, 118.3, 115.2, 54.8, 37.3, 21.3. HRMS (ESI$^+$): calcd. for $C_{11}H_{13}N_2O_3$ [M+H]$^+$: 221.0921, found: 221.0920. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R (*S*) = 12.1 min, t_R (*R*) = 15.2 min (Figure S18).

Rac-2-(3-chlorophenyl)-5-carboxylpyrazolidin-3-one (*rac*-**11e**)



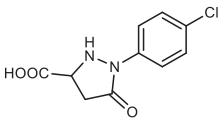
Light yellow solid. 213 mg (71% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.02 (brs, 1H), 7.85 (t, J = 2.1 Hz, 1H), 7.74 (dd, J = 8.3, 2.0 Hz, 1H), 7.38 (t, J = 8.2 Hz, 1H), 7.15 (dd, J = 7.9, 2.1 Hz, 1H), 6.61 (brs, 1H), 4.27 (dd, J = 8.5, 5.8 Hz, 1H), 3.01 (dd, J = 16.6, 8.6 Hz, 1H), 2.78 (dd, J = 16.5, 5.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.2, 170.7, 140.3, 133.0, 130.3, 123.2, 117.2, 116.2, 54.9, 37.3. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3Cl$ [M+H]$^+$: 241.0374, found: 241.0372. Chiral HPLC conditions E: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 1.0 mL/min, rt, UV detection at 260 nm, t_R (*S*) = 13.3 min, t_R (*R*) = 18.0 min (Figure S19).

Rac-2-(4-fluorophenyl)-5-carboxylpyrazolidin-3-one (*rac*-**11f**)



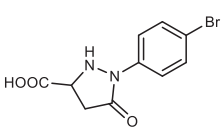
Light yellow solid. 90 mg (74% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.98 (brs, 1H), 7.80 – 7.77 (m, 2H), 7.20 (t, J = 8.9 Hz, 2H), 6.59 (brs, 1H), 4.24 (dd, J = 8.5, 5.8 Hz, 1H), 2.98 (dd, J = 16.4, 8.6 Hz, 1H), 2.75 (dd, J = 16.4, 5.8 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.3, 169.9, 158.2 (d, J = 241.9 Hz), 135.5 (d, J = 2.5 Hz, 2C), 119.8 (d, J = 17.6 Hz), 115.1 (d, J = 22.7 Hz, 2C), 54.8, 37.1. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3F$ $[M+H]^+$: 225.0670, found: 225.0667. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R (S) = 11.2 min, t_R (R) = 13.8 min (Figure S20).

Rac-2-(4-chlorophenyl)-5-carboxylpyrazolidin-3-one (*rac*-**11g**)



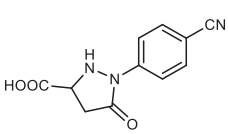
Light yellow solid. 185 mg (57% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.02 (brs, 1H), 7.80 (d, J = 9.0 Hz, 2H), 7.41 (d, J = 9.0 Hz, 2H), 6.57 (brs, 1H), 4.26 (dd, J = 8.5, 5.9 Hz, 1H), 2.99 (dd, J = 16.5, 8.5 Hz, 1H), 2.77 (dd, J = 16.5, 5.9 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.2, 170.4, 137.9, 128.4 (2C), 127.2, 119.4, 119.2, 54.8, 37.2. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3Cl$ $[M+H]^+$: 241.0374, found: 241.0374. Chiral HPLC conditions F: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (25%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 1.0 mL/min, rt, UV detection at 260 nm, t_R (S) = 9.8 min, t_R (R) = 13.3 min (Figure S21).

Rac-2-(4-bromophenyl)-5-carboxylpyrazolidin-3-one (*rac*-**11h**)



Light yellow solid. 224 mg (69% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.02 (brs, 1H), 7.74 (d, J = 9.0 Hz, 2H), 7.54 (d, J = 9.0 Hz, 2H), 6.57 (brs, 1H), 4.25 (dd, J = 8.5, 5.9 Hz, 1H), 2.99 (dd, J = 16.5, 8.6 Hz, 1H), 2.76 (dd, J = 16.5, 5.9 Hz, 1H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.2, 170.4, 138.3, 131.4, 131.3, 119.8, 119.6, 115.2, 54.8, 37.2. HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3Br$ $[M+H]^+$: 284.9869, found: 284.9870. Chiral HPLC conditions F: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (25%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 1.0 mL/min, rt, UV detection at 260 nm, t_R (S) = 14.6 min, t_R (R) = 20.7 min (Figure S22).

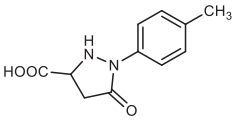
Rac-2-(4-cyanophenyl)-5-carboxylpyrazolidin-3-one (*rac*-**11i**)



Yellow solid. 130 mg (70% yield). ^{1}H NMR (500 MHz, Methanol-d_4): δ 8.05 (d, J = 8.9 Hz, 2H), 7.71 (d, J = 8.9 Hz, 2H), 4.34 (dd, J = 8.5, 6.7 Hz, 1H), 3.11 (dd, J = 16.8, 8.5 Hz, 1H), 2.96 (dd, J = 16.8, 6.7 Hz, 1H); ^{13}C NMR (126 MHz, Methanol-d_4): δ 173.7, 173.2, 143.9, 134.0,

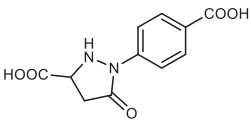
133.9, 119.8, 119.5 (2C), 107.85, 56.5, 38.8. HRMS (ESI$^+$): calcd. for $C_{11}H_{10}N_3O_3$ $[M+H]^+$: 232.0717, found: 232.0713. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R (peak-1) = 19.1 min, t_R (peak-2) = 22.0 min (Figure S23).

Rac-2-(4-methylphenyl)-5-carboxylpyrazolidin-3-one (*rac*-**11j**)



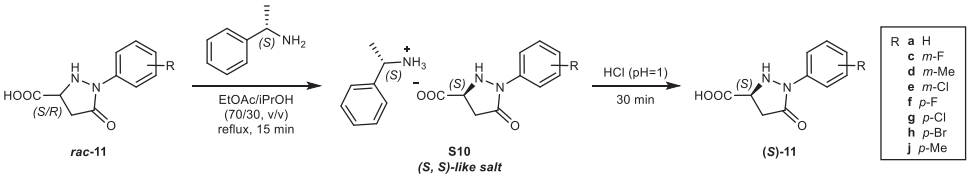
Orange solid. 238 mg (57% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.98 (brs, 1H), 7.65 (d, J = 8.3 Hz, 2H), 7.15 (d, J = 8.3 Hz, 2H), 6.49 (brs, 1H), 4.22 (dd, J = 8.5, 5.9 Hz, 1H), 2.96 (dd, J = 16.4, 8.6 Hz, 1H), 2.75 (dd, J = 16.4, 5.9 Hz, 1H), 2.27 (s, 3H); ^{13}C NMR (126 MHz, DMSO-d_6): δ 172.3, 169.7, 136.6, 132.6, 128.9, 128.8, 118.0, 117.8, 54.8, 37.2, 20.5. HRMS (ESI$^+$): calcd. for $C_{11}H_{13}N_2O_3$ $[M+H]^+$: 221.0921, found: 221.0918. Chiral HPLC conditions G: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (25%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R (*S*) = 11.0 min, t_R (*R*) = 14.4 min (Figure S24).

Rac-2-(4-carboxylphenyl)-5-carboxylpyrazolidin-3-one (*rac*-**11k**)



Orange solid. 75 mg (53% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.91 (brs, 2H), 7.94 (d, J = 8.9 Hz, 2H), 7.89 (d, J = 8.9 Hz, 2H), 6.63 (brs, 1H), 4.28 (dd, J = 8.5, 6.0 Hz, 1H), 3.02 (dd, J = 16.6, 8.5 Hz, 1H), 2.80 (dd, J = 16.6, 5.9 Hz, 1H); ^{13}C NMR (126 MHz, DMSO): δ 172.1, 171.0, 166.9, 142.5, 130.2, 130.1, 125.2, 117.1, 116.9, 54.8, 37.4. HRMS (ESI$^+$): calcd. for $C_{11}H_{11}N_2O_5$ $[M+H]^+$: 251.0662, found: 251.0659.

11. Optical resolution of 2-aryl-5-carboxylpyrazolidin-3-ones

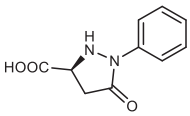


General procedure. The optical resolution procedure was performed according to a procedure published elsewhere.[1] The *rac*-2-aryl-5-carboxylpyrazolidin-3-one (*rac*-**11a**, 103 mg, 0.5 mmol; *rac*-**11c**, 112 mg, 0.5 mmol; *rac*-**11d**, 110 mg, 0.5 mmol; *rac*-**11e**, 120 mg, 0.5 mmol; *rac*-**11f**, 46 mg, 0.2 mmol; *rac*-**11g**, 120 mg, 0.5 mmol; *rac*-**11h**, 142 mg, 0.5 mmol; *rac*-**11j**, 110 mg, 0.5 mmol) was dissolved in EtOAc/i-PrOH (70/30, v/v, 2 mL) and the mixture was

heated to reflux until the solution became homogeneous. The (*S*)-α-methylbenzylamine (half equivalent) was added and the reaction mixture was heated to reflux until the expected (*S*, *S*)-like salt (**S10**) precipitates. The reaction mixture was allowed to cool down to room temperature and then kept in an ice-bath for 30 min. The white precipitate was filtered off, washed with cold EtOAc (0.5 mL) and dried under vacuum overnight.

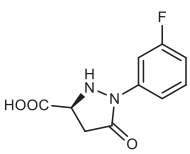
The (*S*, *S*)-like salt (**S10**) was dissolved in water (1 mL) and the pH was adjusted to 2.0 with HCl (1 M). The solution was stirred in an ice-bath until precipitation of the (*S*)-enriched desired product [(S)-**11**)]. The (*S*)-enriched product was filtered off, washed with cold water and dried under vacuum overnight.

(*S*)-2-phenyl-5-carboxylpyrazolidin-3-one [(*S*)-**11a**]



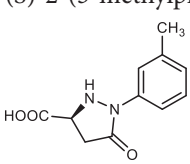
White solid. 27 mg (26% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.96 (brs, 1H), 7.78 – 7.76 (m, 2H), 7.36 – 7.33 (m, 2H), 7.09 (tt, J = 7.4, 1.2 Hz, 1H), 6.54 (brs, 1H), 4.24 (dd, J = 8.5, 5.9 Hz, 1H), 2.98 (dd, J = 16.4, 8.6 Hz, 1H), 2.76 (dd, J = 16.4, 5.8 Hz, 1H). The NMR data are in agreement with published data.[1] HRMS (ESI$^+$): calcd. for $C_{10}H_{11}N_2O_3$ [M+H]$^+$: 207.0764, found: 207.0763. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R (*S,* major) = 9.2 min, t_R (*R,* minor) = 11.3 min. The *ee* was determined to be 92.7% by chiral HPLC analysis using racemic standard (Figure S15).

(*S*)-2-(3-fluorophenyl)-5-carboxylpyrazolidin-3-one [(*S*)-**11c**]



White solid. 25 mg (22% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.06 (brs, 1H), 7.64 – 7.61 (m, 2H), 7.43 – 7.38 (m, 2H), 6.95 – 6.92 (m, 1H), 6.61 (brs, 1H), 4.27 (dd, J = 8.5, 5.8 Hz, 1H), 3.02 (dd, J = 16.5, 8.6 Hz, 1H), 2.79 (dd, J = 16.6, 5.7 Hz, 1H). HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3F$ [M+H]$^+$: 225.0670, found: 225.0666. Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R (*S,* major) = 14.4 min, t_R (*R,* minor) = 17.9 min. The *ee* was determined to be 93.9% by chiral HPLC analysis using racemic standard (Figure S17).

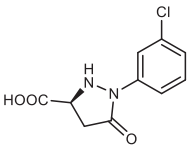
(*S*)-2-(3-methylphenyl)-5-carboxylpyrazolidin-3-one [(*S*)-**11d**]



White solid. 11 mg (10% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.97 (brs, 1H), 7.60 – 7.58 (m, 2H), 7.22 (t, J = 7.8 Hz, 1H), 6.91 (d, J = 7.5 Hz, 1H), 6.50 (brs, 1H), 4.22 (dd, J = 8.6, 5.9 Hz, 1H), 2.96 (dd, J = 16.4, 8.6 Hz, 1H), 2.75 (dd, J = 16.4, 5.8 Hz, 1H), 2.29 (s, 3H). Chiral

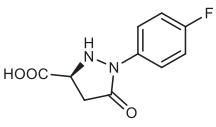
HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R (*S,* major) = 11.9 min, t_R (*R,* minor) = 14.9 min. The *ee* was determined to be 33.6% by chiral HPLC analysis using racemic standard (Figure S18).

(*S*)-2-(3-chlorophenyl)-5-carboxylpyrazolidin-3-one [(*S*)-**11e**]



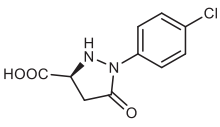
White solid. 20 mg (17% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.84 (t, J = 2.1 Hz, 1H), 7.74 (dd, J = 8.3, 2.0 Hz, 1H), 7.38 (t, J = 8.2 Hz, 1H), 7.15 (dd, J = 8.0, 2.1 Hz, 1H), 4.25 (dd, J = 8.5, 5.9 Hz, 1H), 3.00 (dd, J = 16.6, 8.5 Hz, 1H), 2.77 (dd, J = 16.5, 5.9 Hz, 1H). HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3Cl$ [M+H]$^+$: 241.0374, found: 241.0372. Chiral HPLC conditions E: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 1.0 mL/min, rt, UV detection at 260 nm, t_R (*S,* major) = 13.1 min, t_R (*R,* minor) = 17.9 min. The *ee* was determined to be 86.7% by chiral HPLC analysis using racemic standard (Figure S19).

(*S*)-2-(4-fluorophenyl)-5-carboxylpyrazolidin-3-one [(*S*)-**11f**]



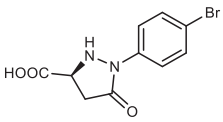
White solid. 9 mg (19% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 12.95 (brs, 1H), 7.79 – 7.77 (m, 2H), 7.20 (t, J = 9.0 Hz, 2H), 6.61 (brs, 1H), 4.24 (dd, J = 8.6, 5.9 Hz, 1H), 2.98 (dd, J = 16.4, 8.6 Hz, 1H), 2.75 (dd, J = 16.4, 5.9 Hz, 1H). Chiral HPLC conditions C: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R (*S,* major) = 11.2 min, t_R (*R,* minor) = 13.9 min. The *ee* was determined to be 97.7% by chiral HPLC analysis using racemic standard (Figure S20).

(*S*)-2-(4-chlorophenyl)-5-carboxylpyrazolidin-3-one [(*S*)-**11g**]



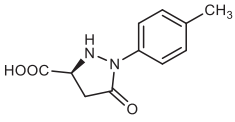
White solid. 28 mg (23% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.03 (brs, 1H), 7.79 (d, J = 8.9 Hz, 2H), 7.41 (d, J = 9.0 Hz, 2H), 6.59 (brs, 1H), 4.26 (d, J = 8.7 Hz, 1H), 2.99 (dd, J = 16.5, 8.5 Hz, 1H), 2.76 (dd, J = 16.5, 5.8 Hz, 1H). HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3Cl$ [M+H]$^+$: 241.0374, found: 241.0372. Chiral HPLC conditions F: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (25%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 1.0 mL/min, rt, UV detection at 260 nm, t_R (*S,* major) = 9.8 min, t_R (*R,* minor) = 13.3 min. The *ee* was determined to be 88.7% by chiral HPLC analysis using racemic standard (Figure S21).

(*S*)-2-(4-bromophenyl)-5-carboxylpyrazolidin-3-one [(*S*)-**11h**]



White solid. 42 mg (29% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.02 (brs, 1H), 7.74 (d, J = 9.0 Hz, 2H), 7.54 (d, J = 9.0 Hz, 2H), 6.58 (brs, 1H), 4.25 (brs, 1H), 2.98 (dd, J = 16.5, 8.5 Hz, 1H), 2.76 (dd, J = 16.6, 5.9 Hz, 1H). HRMS (ESI$^+$): calcd. for $C_{10}H_{10}N_2O_3Br$ $[M+H]^+$: 284.9869, found: 284.9868. Chiral HPLC conditions F: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (25%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 1.0 mL/min, rt, UV detection at 260 nm, t_R (*S,* major) = 14.6 min, t_R (*R,* minor) = 20.8 min. The *ee* was determined to be 89.6% by chiral HPLC analysis using racemic standard (Figure S22).

(*S*)-2-(4-methylphenyl)-5-carboxylpyrazolidin-3-one [(*S*)-**11j**]



Light yellow solid. 28 mg (25% yield). ^{1}H NMR (500 MHz, DMSO-d_6): δ 13.00 (s, 1H), 7.65 (d, J = 8.5 Hz, 2H), 7.15 (d, J = 8.3 Hz, 2H), 6.51 (s, 1H), 4.22 (dd, J = 8.6, 5.9 Hz, 1H), 2.95 (dd, J = 16.4, 8.6 Hz, 1H), 2.74 (dd, J = 16.4, 5.9 Hz, 1H), 2.26 (s, 3H). HRMS (ESI$^+$): calcd. for $C_{11}H_{13}N_2O_3$ $[M+H]^+$: 221.0921, found: 221.0918. Chiral HPLC conditions G: CHIRALPAK AD-RH column with isocratic MeCN/H_2O (25%, v/v, 0.1% formic acid) as mobile phase with a flow rate of 0.5 mL/min, rt, UV detection at 260 nm, t_R (*S,* major) = 11.0 min, t_R (*R,* minor) = 14.6 min. The *ee* was determined to be 95.2% by chiral HPLC analysis using racemic standard (Figure S24).

III) Chiral HPLC analysis

HPLC condition A: Nucleosil Chiral-1 column (250 x 4 mm, 5 μm) with 0.5 mM $CuSO_4$ aqueous solution as mobile phase at a flow rate of 1.0 mL/min, 60 °C, UV detection at 260 nm.

HPLC condition B: Nucleosil Chiral-1 column (250 x 4 mm, 5 μm) with 0.5 mM $CuSO_4$ aqueous solution as mobile phase at a flow rate of 1.2 mL/min, 60 °C, UV detection at 260 nm.

HPLC condition C: CHIRALPAK AD-RH column (150 x 4.6 mm, 5 μm) with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase at a flow rate of 0.5 mL/min, rt, UV detection at 260 nm.

HPLC condition D: CHIRALPAK AD-RH column (150 x 4.6 mm, 5 μm) with isocratic MeCN/H_2O (15%, v/v, 0.1% TFA) as mobile phase at a flow rate of 0.25 mL/min, rt, UV detection at 260 nm.

HPLC condition E: CHIRALPAK AD-RH column (150 x 4.6 mm, 5 μm) with isocratic MeCN/H_2O (20%, v/v, 0.1% formic acid) as mobile phase at a flow rate of 1.0 mL/min, rt, UV detection at 260 nm.

HPLC condition F: CHIRALPAK AD-RH column (150 x 4.6 mm, 5 μm) with isocratic MeCN/H_2O (25%, v/v, 0.1% formic acid) as mobile phase at a flow rate of 1.0 mL/min, rt, UV detection at 260 nm.

HPLC condition G: CHIRALPAK AD-RH column (150 x 4.6 mm, 5 μm) with isocratic MeCN/H_2O (25%, v/v, 0.1% formic acid) as mobile phase at a flow rate of 0.5 mL/min, rt, UV detection at 260 nm.

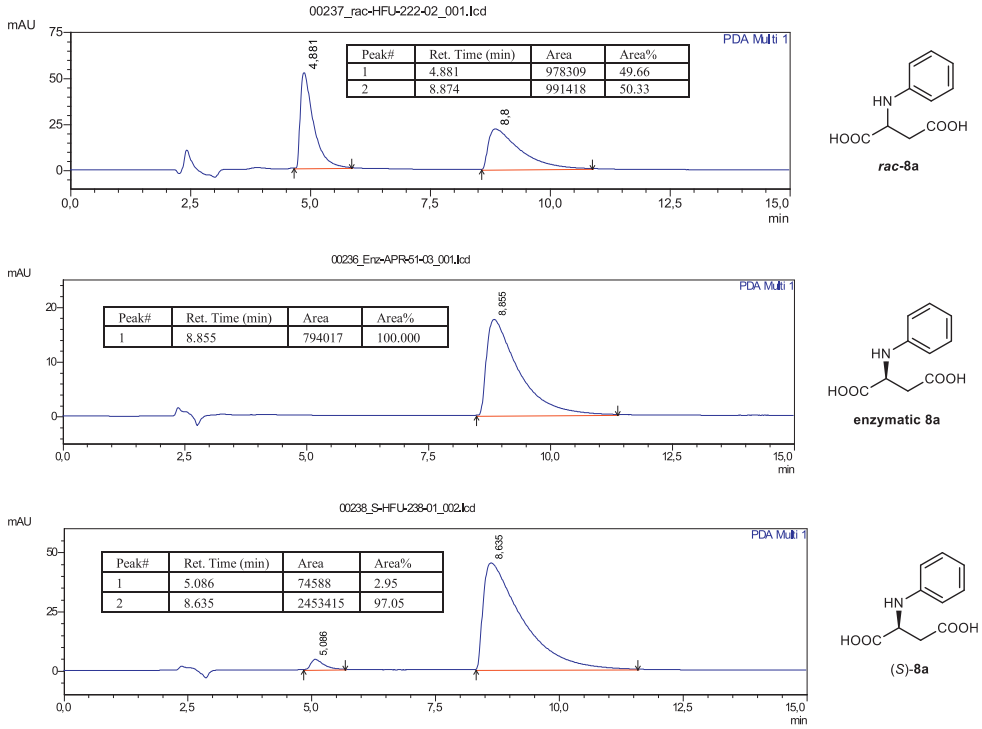


Figure S2. Chiral HPLC analysis of enzymatic product **8a** using HPLC conditions A.

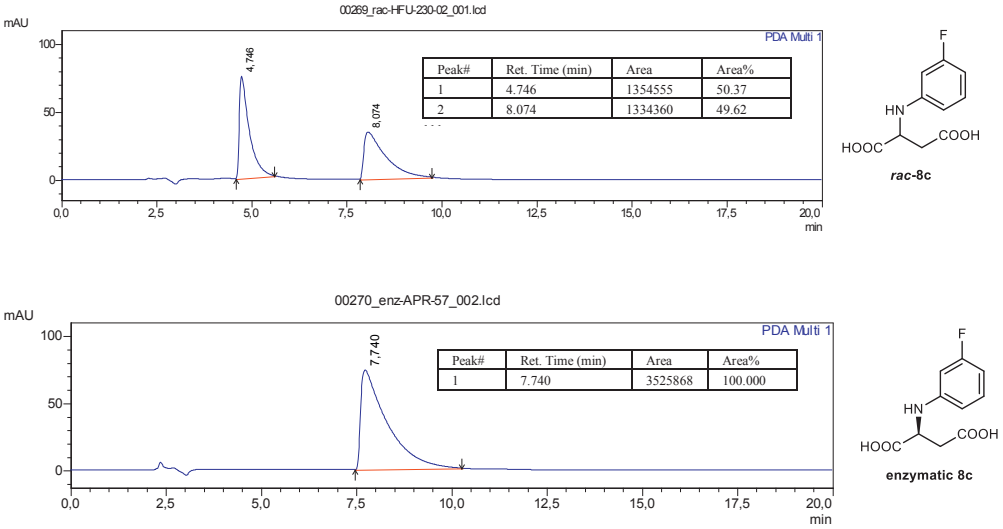


Figure S3. Chiral HPLC analysis of enzymatic product **8c** using HPLC conditions A.

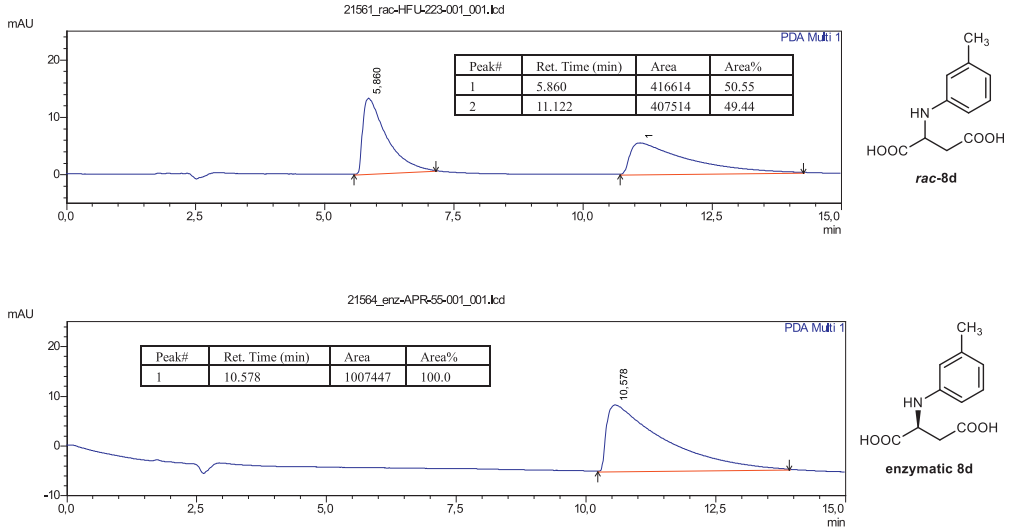


Figure S4. Chiral HPLC analysis of enzymatic product **8d** using HPLC conditions B.

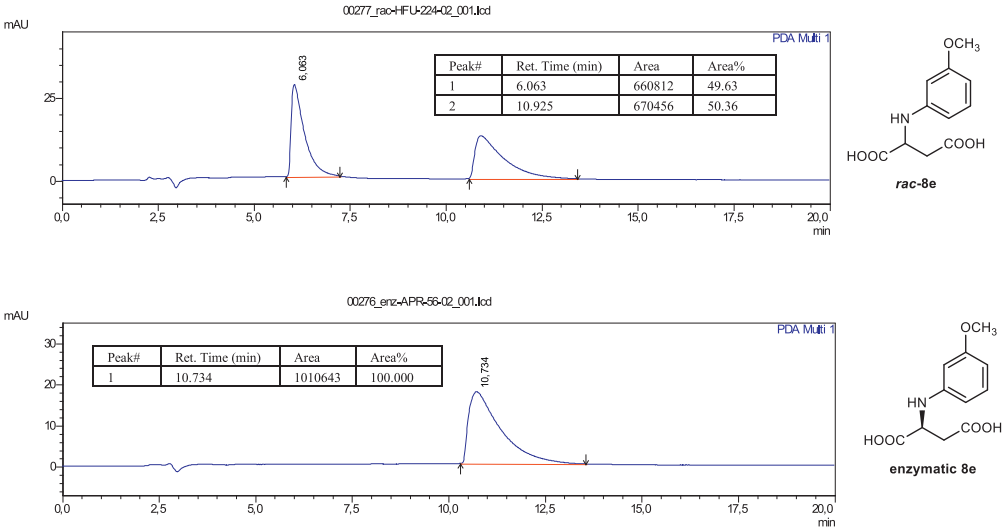


Figure S5. Chiral HPLC analysis of enzymatic product **8e** using HPLC conditions A.

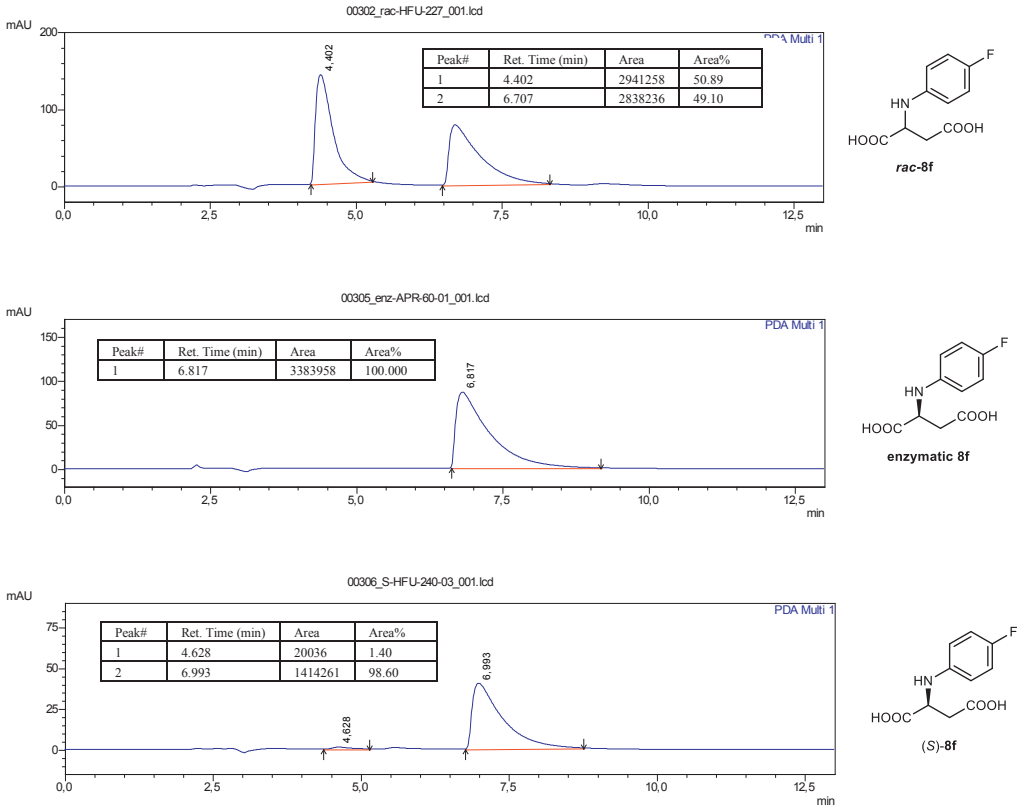


Figure S6. Chiral HPLC analysis of enzymatic product **8f** using HPLC conditions A.

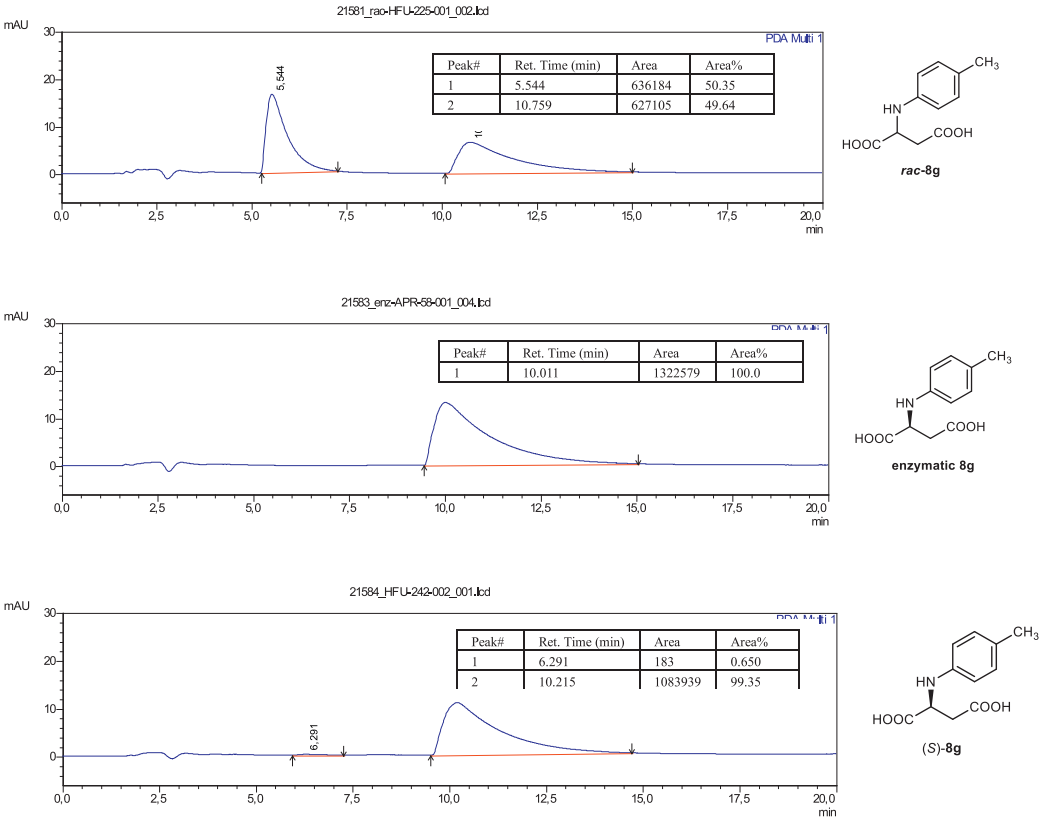


Figure S7. Chiral HPLC analysis of enzymatic product **8g** using HPLC conditions B.

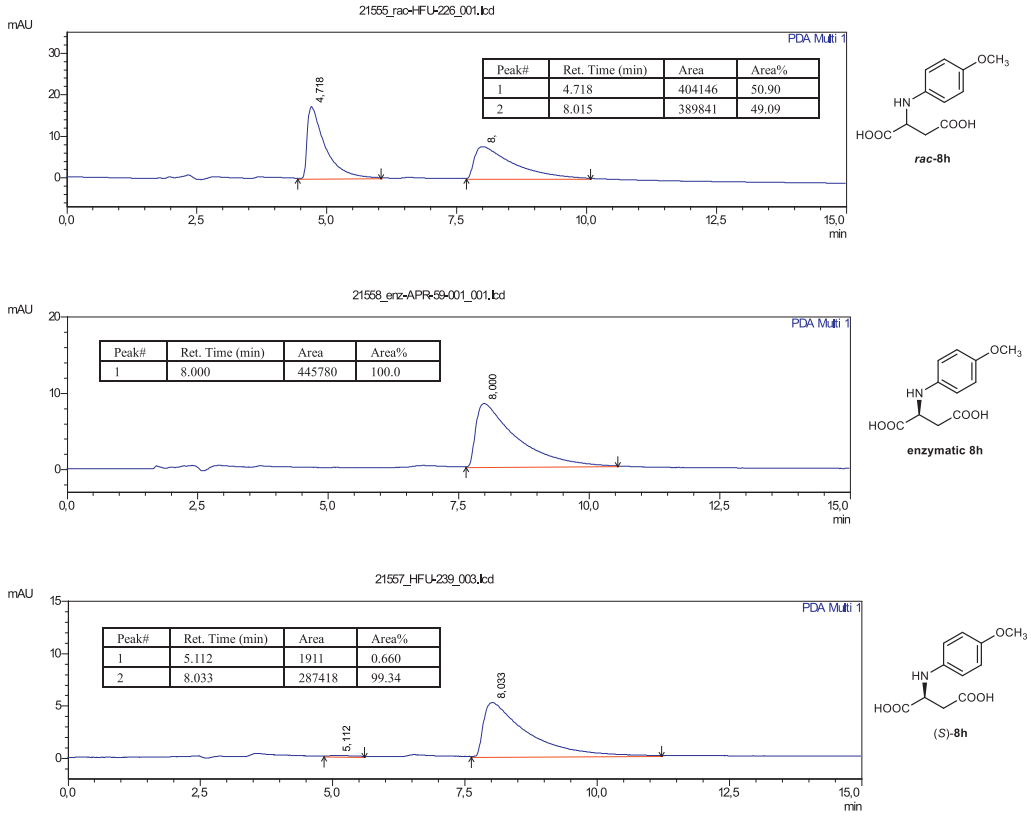


Figure S8. Chiral HPLC analysis of enzymatic product **8h** using HPLC conditions B.

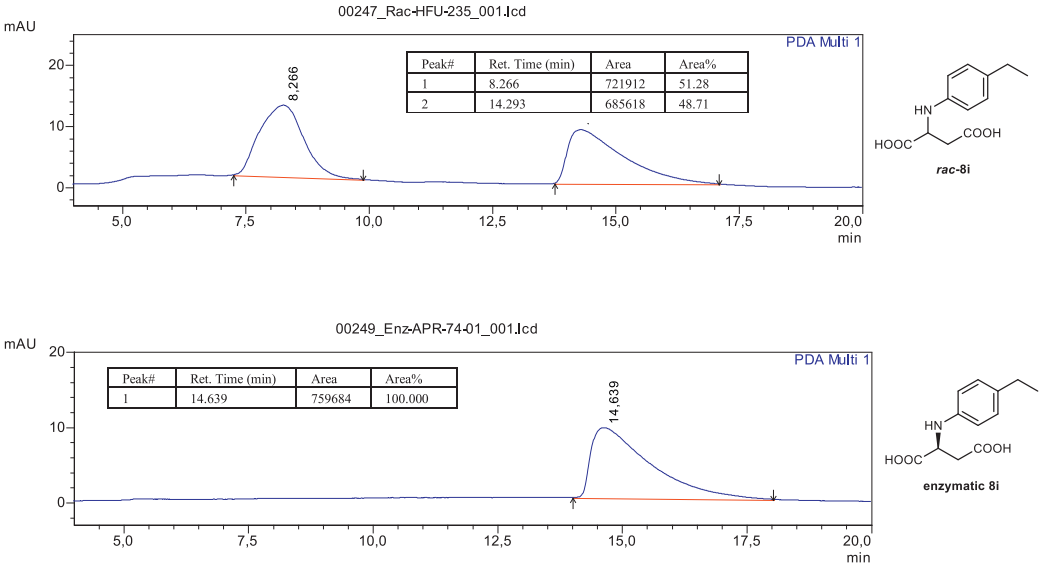


Figure S9. Chiral HPLC analysis of enzymatic product **8i** using HPLC conditions A.

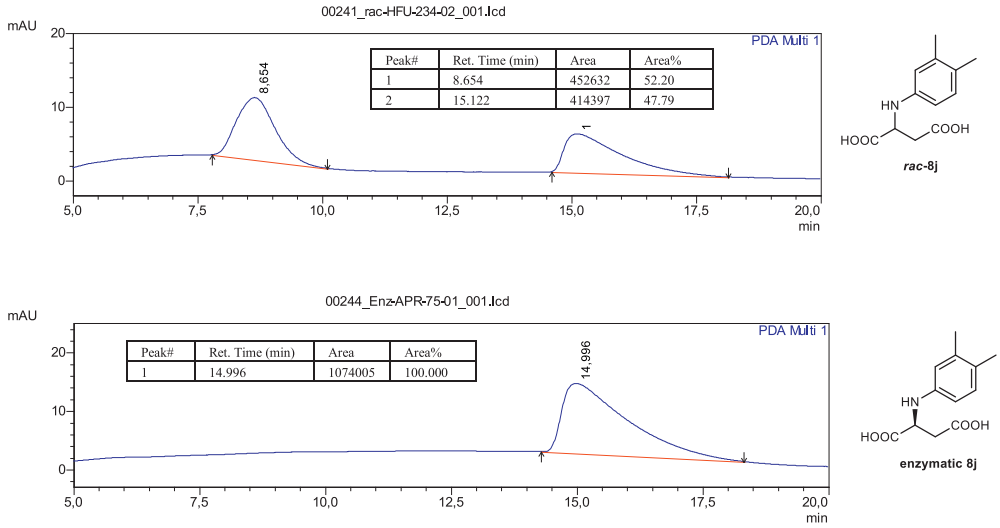


Figure S10. Chiral HPLC analysis of enzymatic product **8j** using HPLC conditions A.

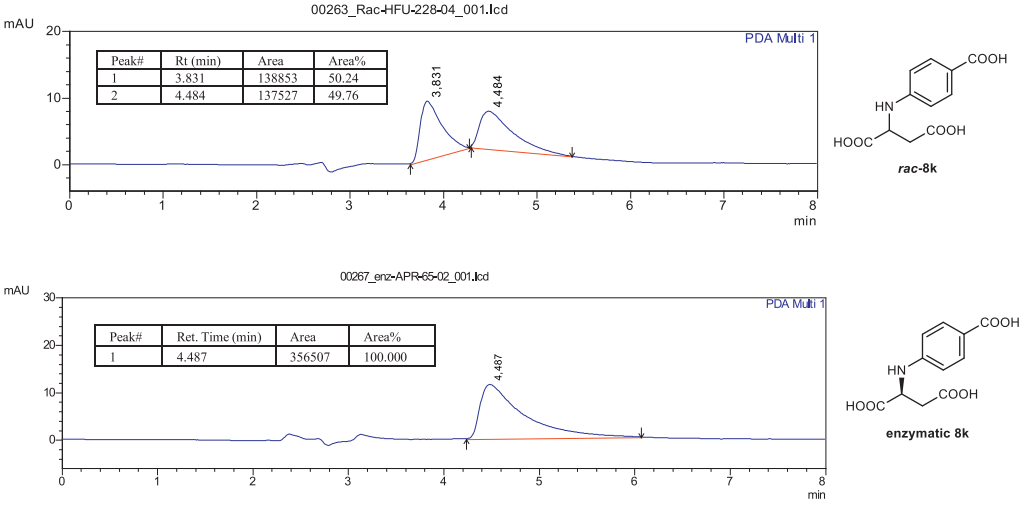


Figure S11. Chiral HPLC analysis of enzymatic product **8k** using HPLC conditions A.

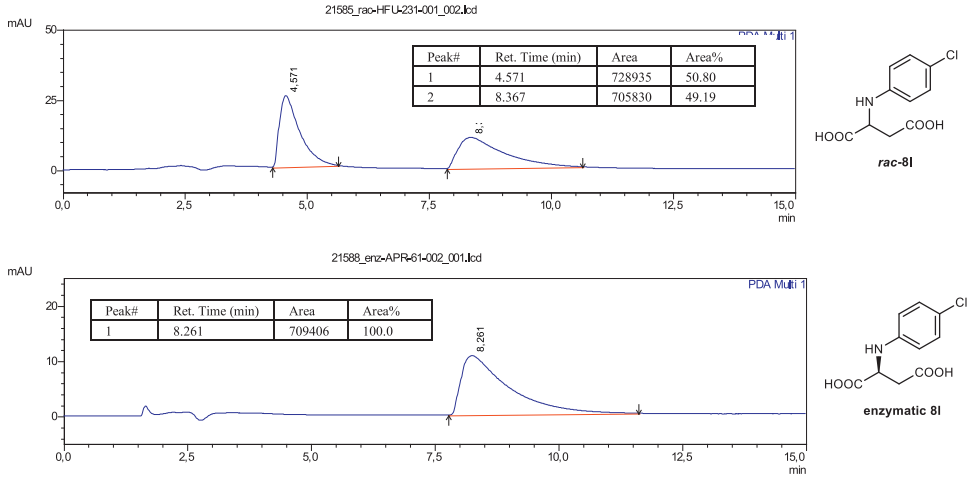


Figure S12. Chiral HPLC analysis of enzymatic product **8l** using HPLC conditions B.

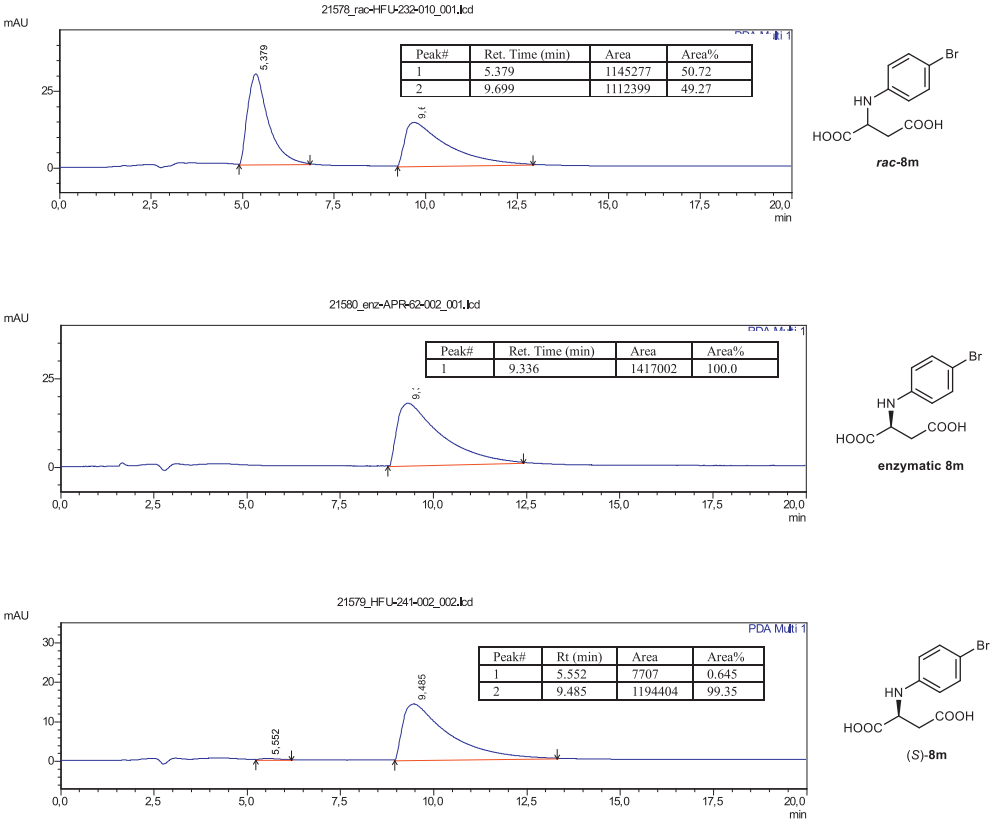


Figure S13. Chiral HPLC analysis of enzymatic product **8m** using HPLC conditions B.

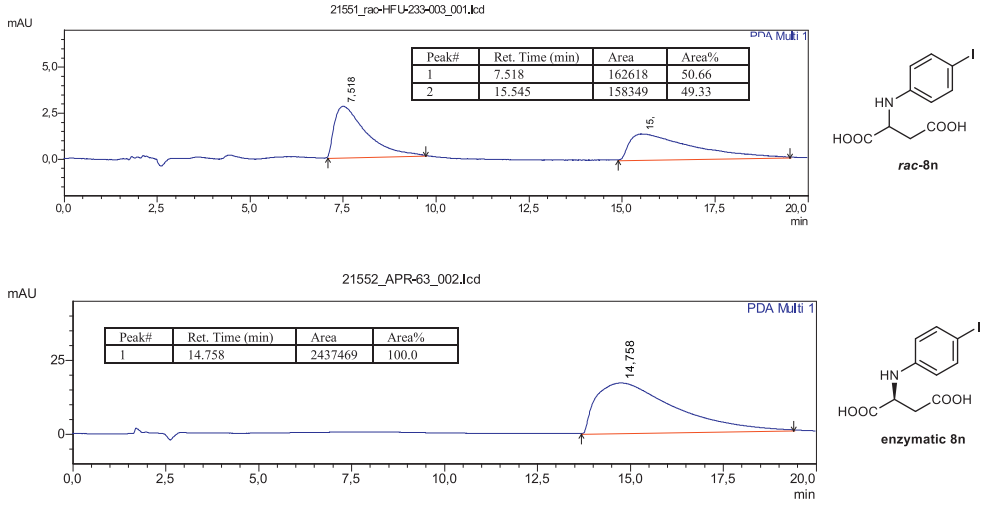


Figure S14. Chiral HPLC analysis of enzymatic product **8n** using HPLC conditions B.

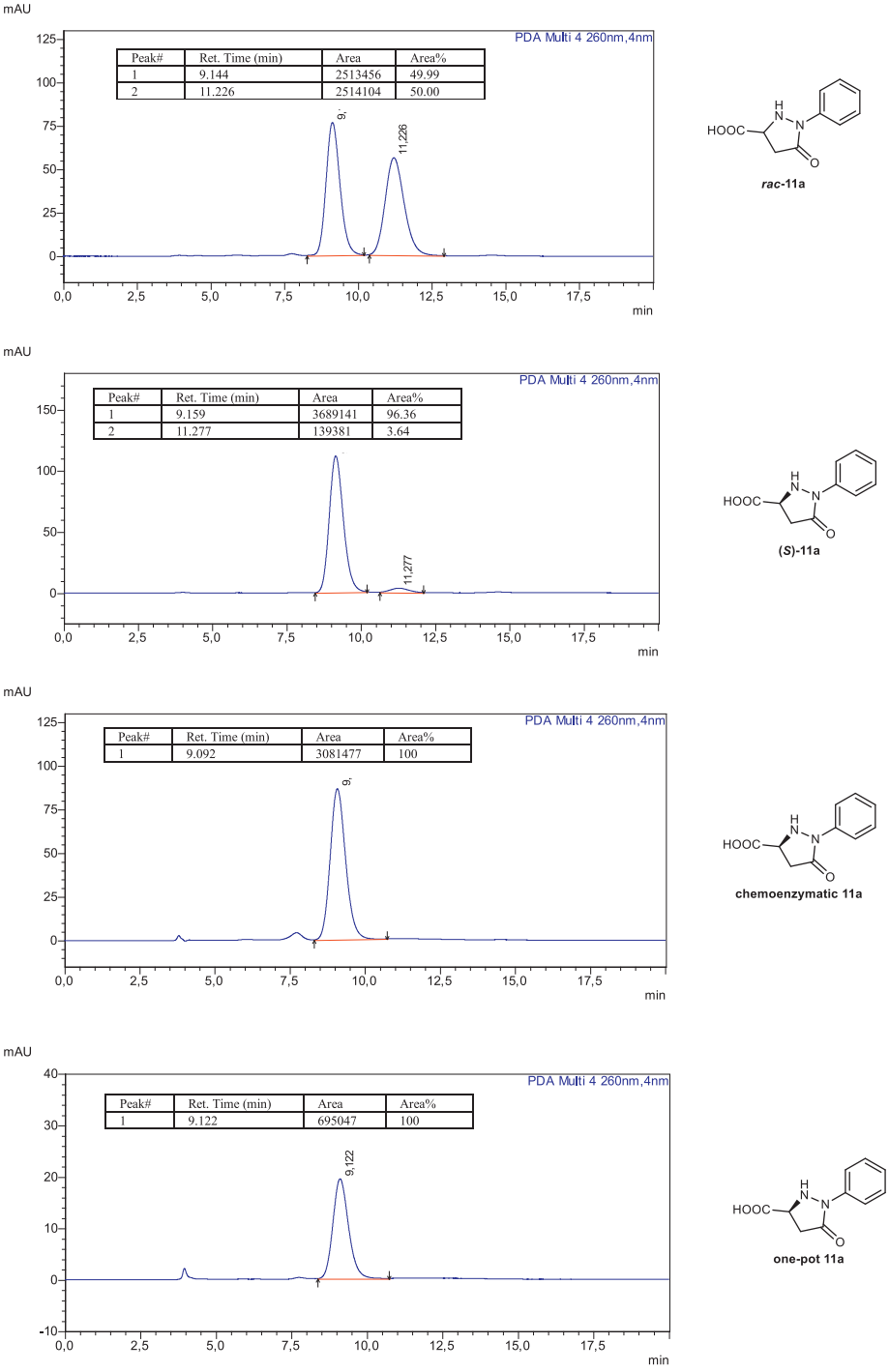


Figure S15. Chiral HPLC analysis of chemoenzymatic product **11a** using HPLC condition C.

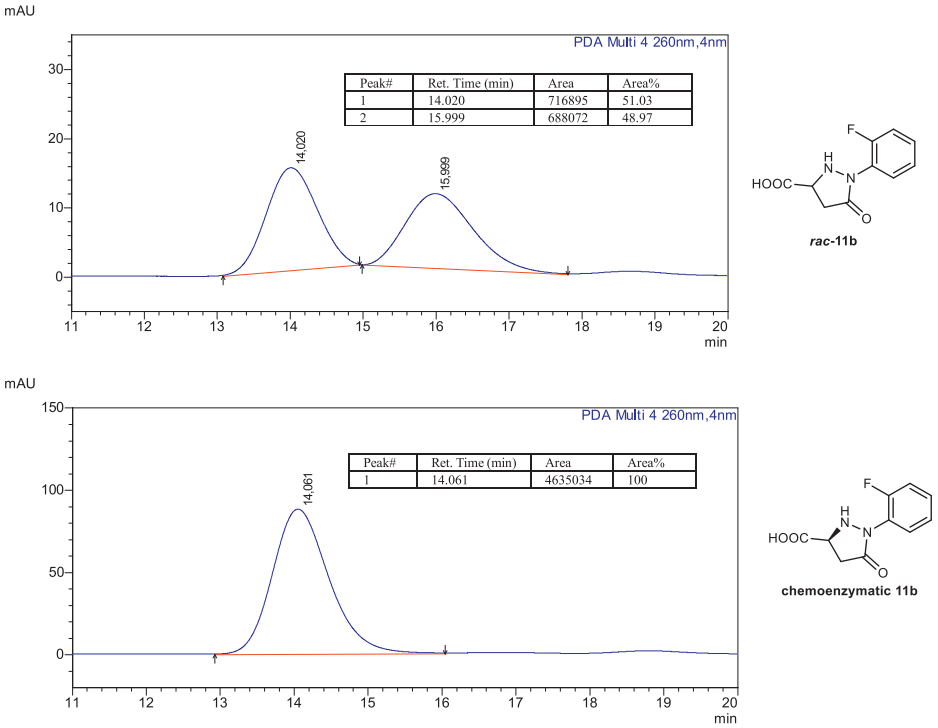


Figure S16. Chiral HPLC analysis of chemoenzymatic product **11b** using HPLC condition D.

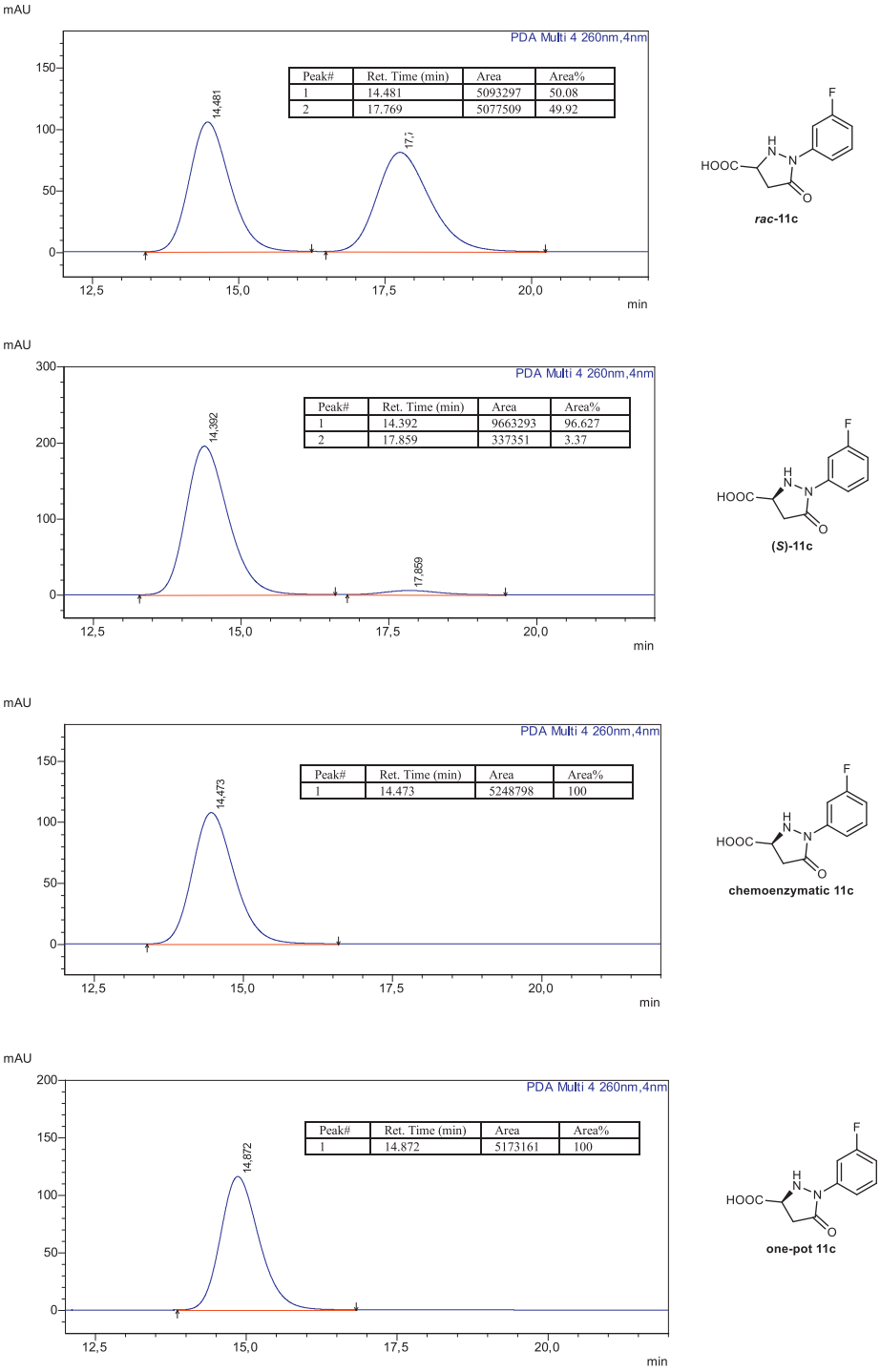


Figure S17. Chiral HPLC analysis of chemoenzymatic product **11c** using HPLC condition C.

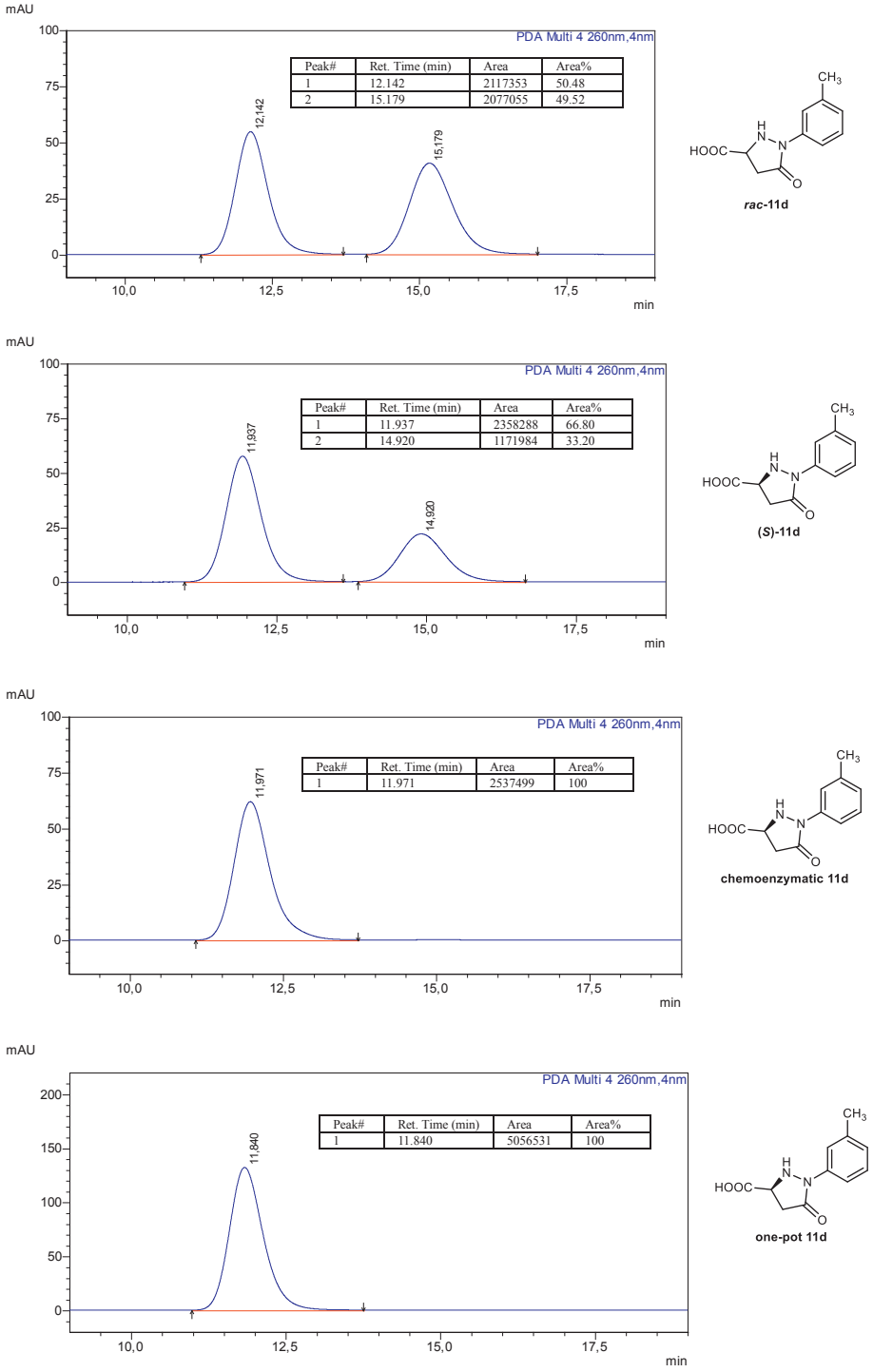


Figure S18. Chiral HPLC analysis of chemoenzymatic product **11d** using HPLC condition C.

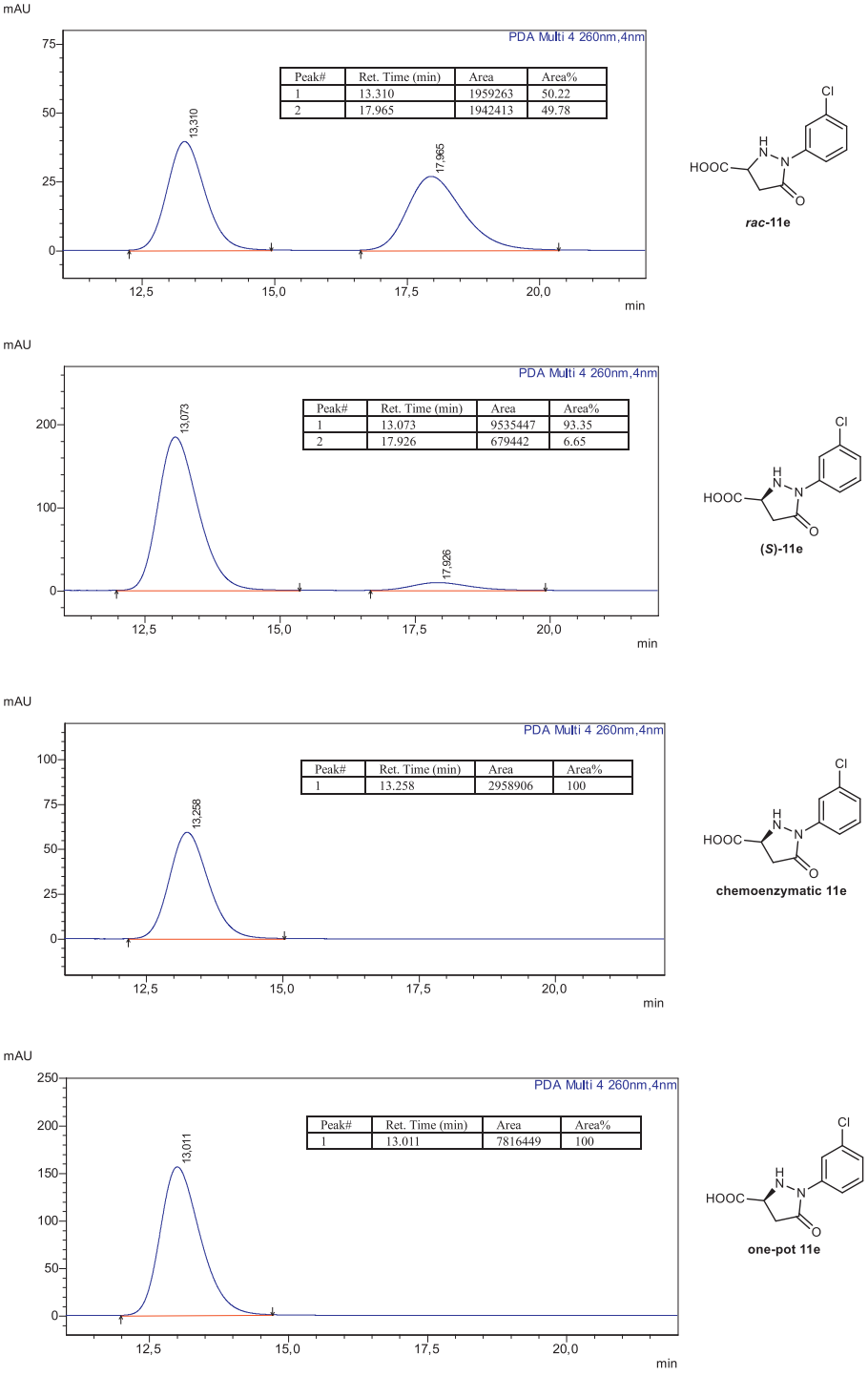


Figure S19. Chiral HPLC analysis of chemoenzymatic product **11e** using HPLC condition E.

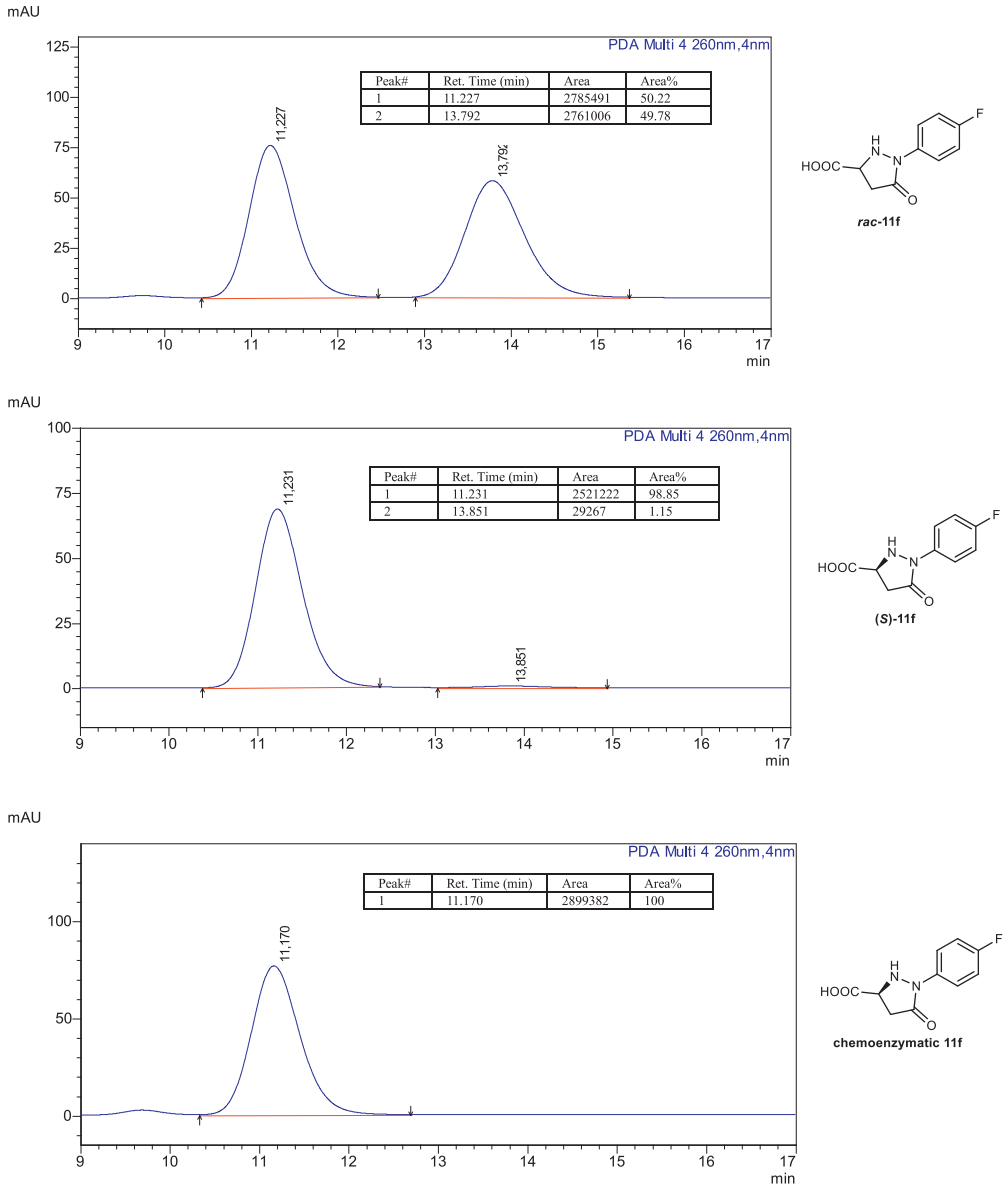


Figure S20. Chiral HPLC analysis of chemoenzymatic product **11f** using HPLC condition C.

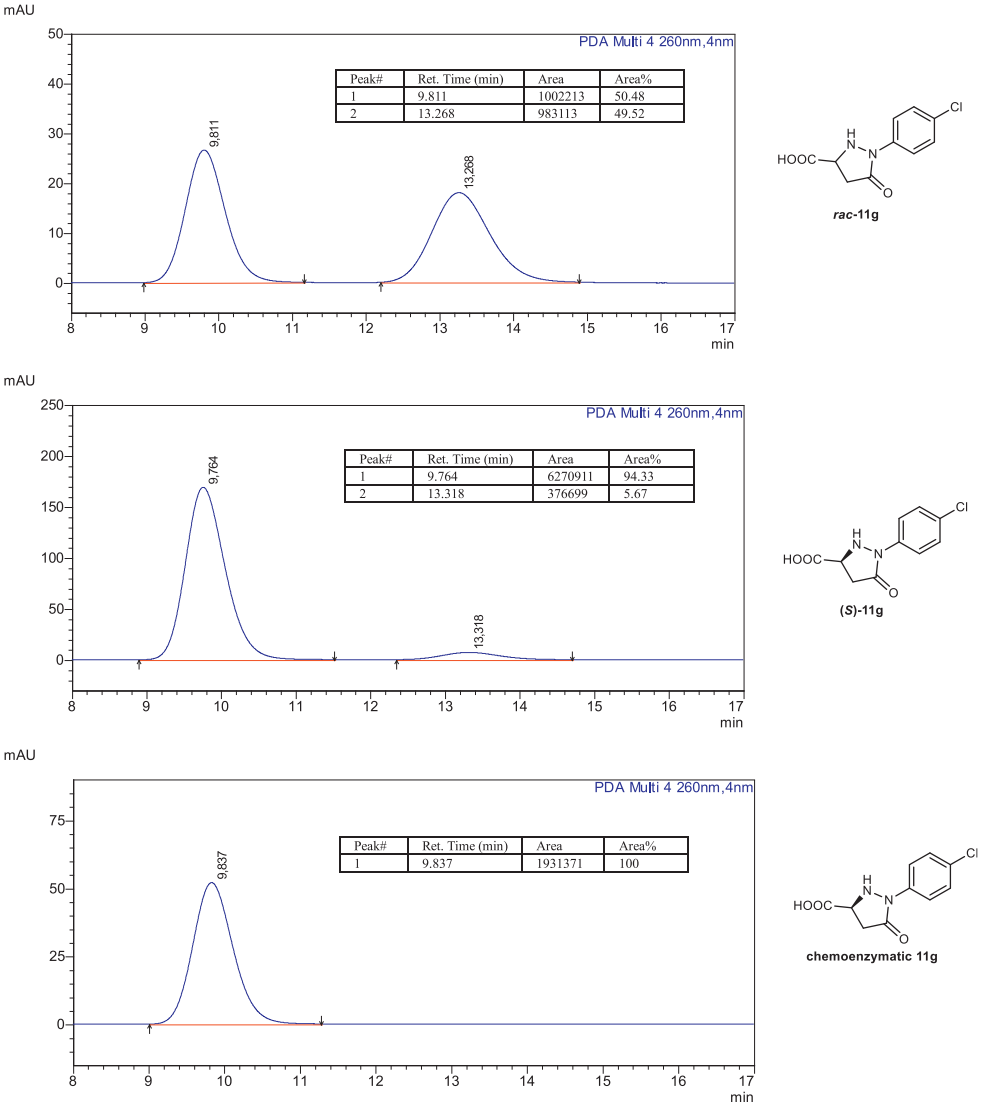


Figure S21. Chiral HPLC analysis of chemoenzymatic product **11g** using HPLC condition F.

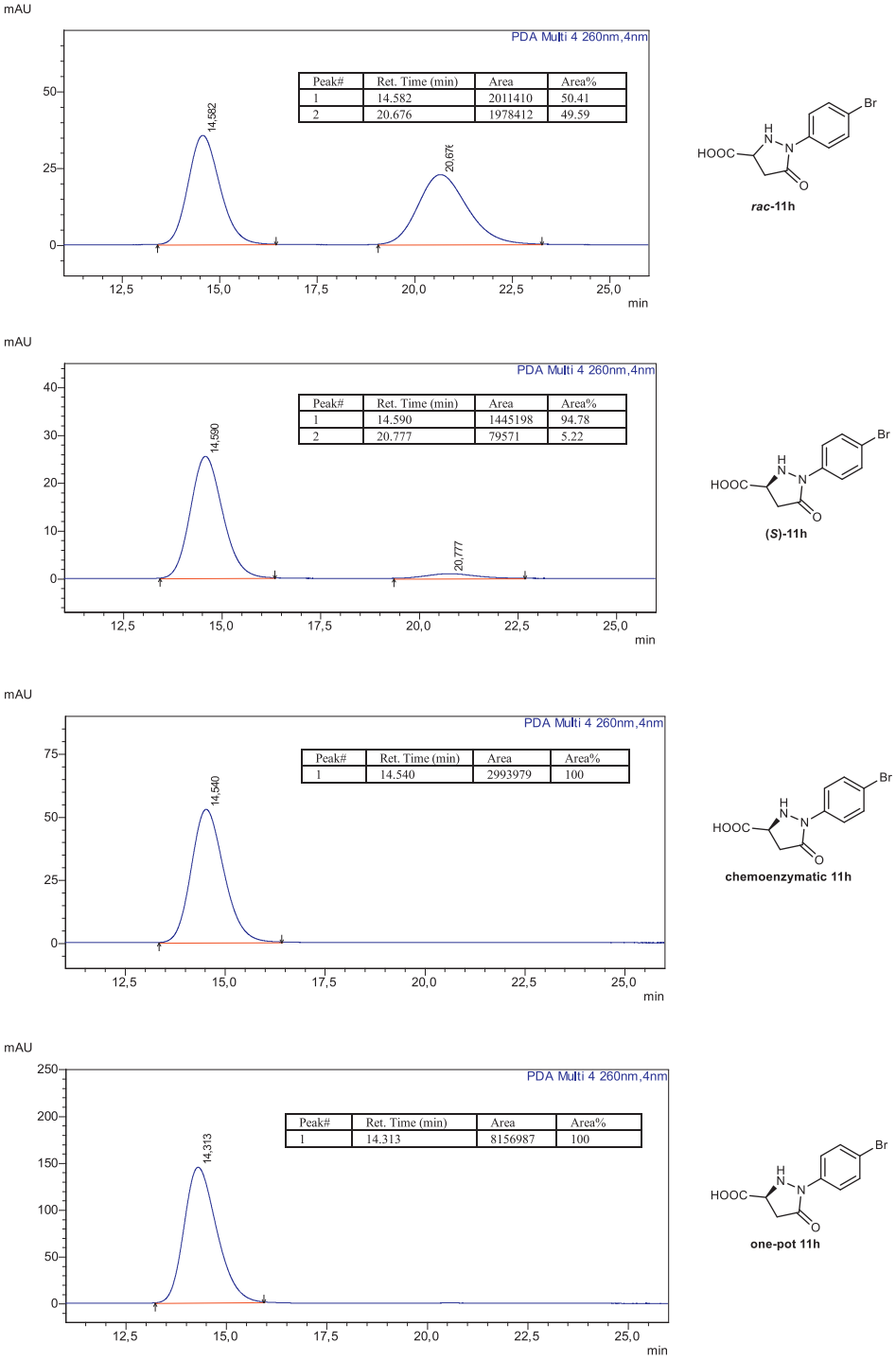


Figure S22. Chiral HPLC analysis of chemoenzymatic product **11h** using HPLC condition F.

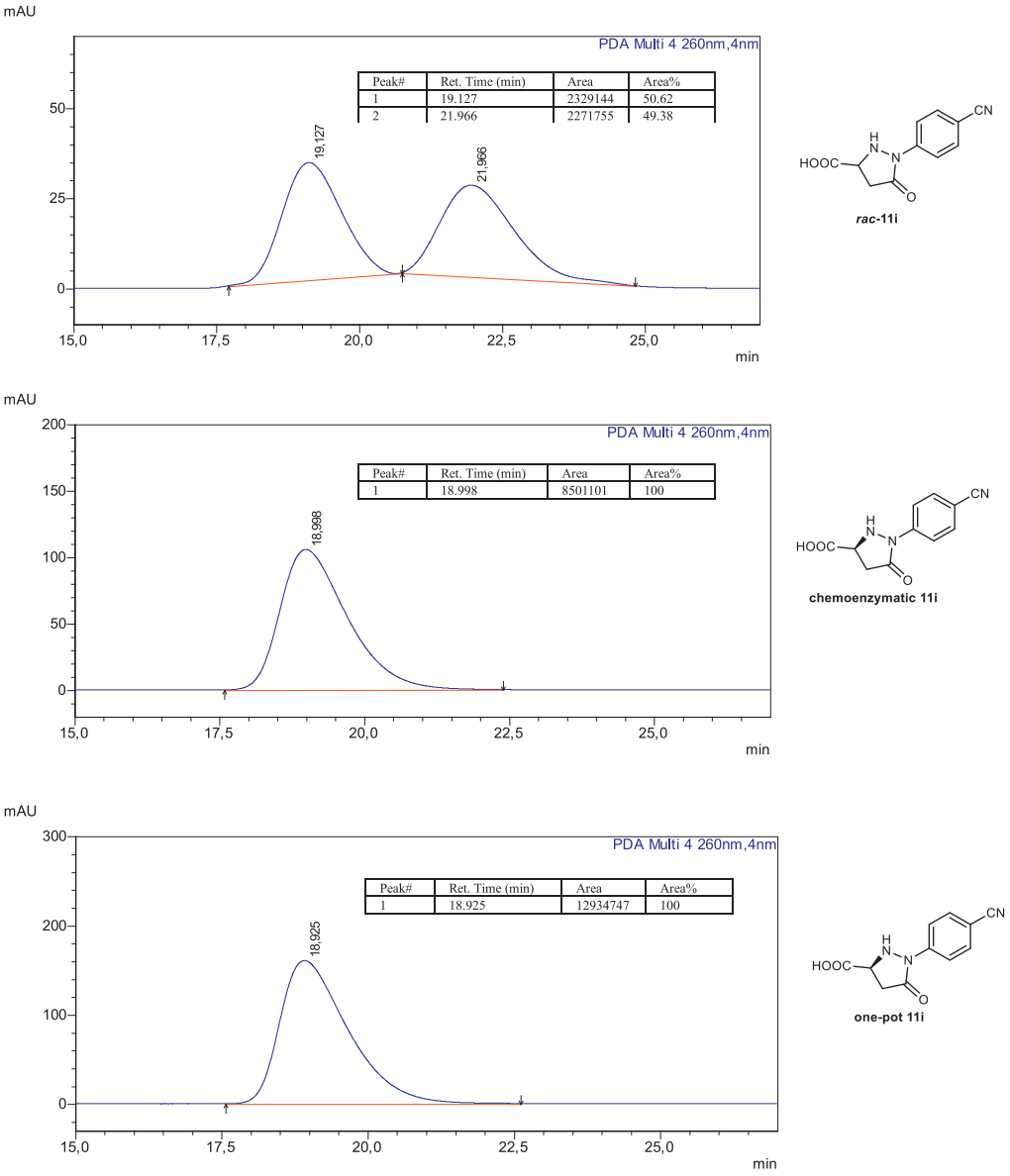


Figure S23. Chiral HPLC analysis of chemoenzymatic product **11i** using HPLC condition C.

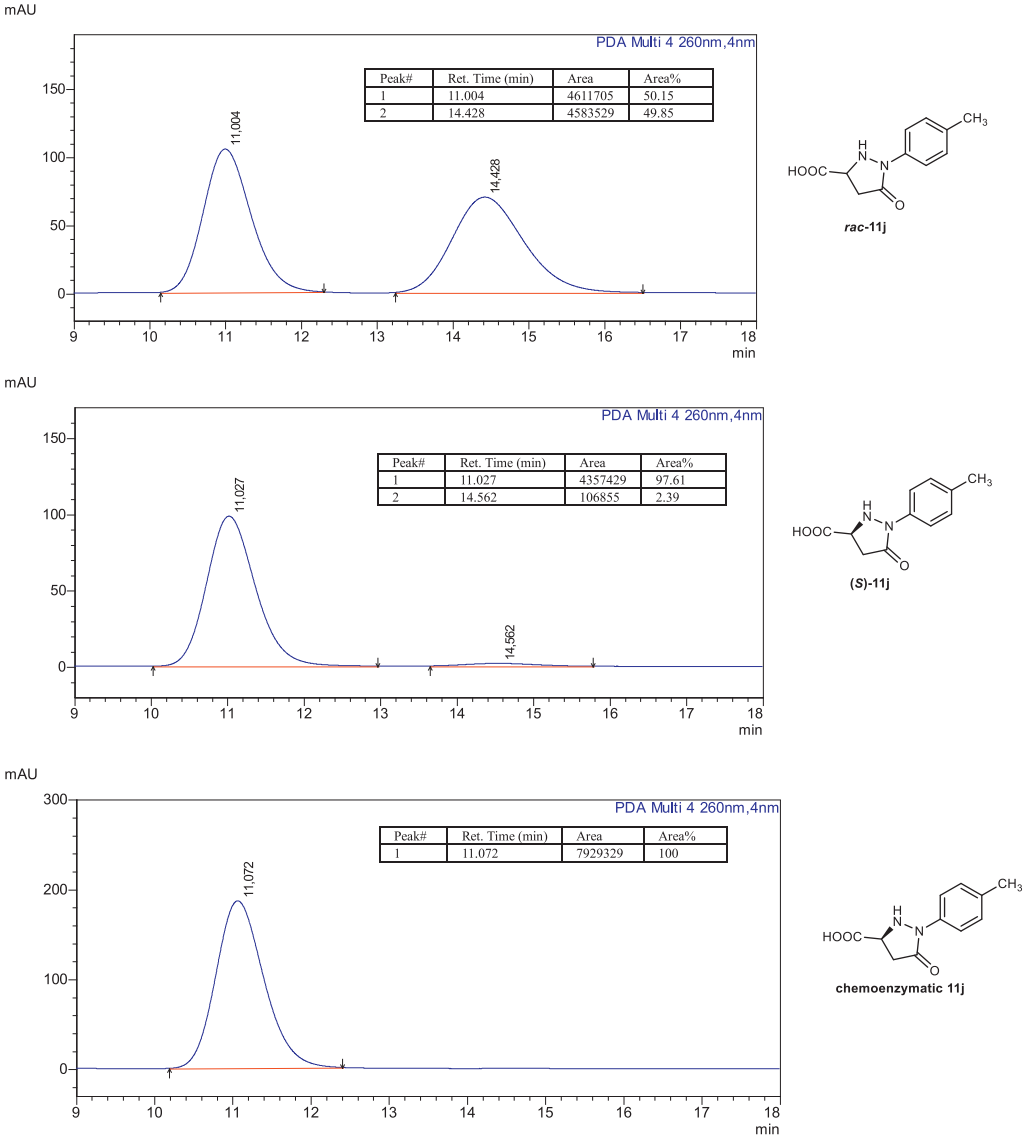


Figure S24. Chiral HPLC analysis of chemoenzymatic product **11j** using HPLC condition G.

IV) Supplementary references

1. Cynthia, A. *et al.* Resolution of 5-oxo-1-phenylpyrazolidine-3-carboxylic acid and synthesis of novel enantiopure amide derivatives. *ARKIVOC* (viii) 55-75 (2010).
2. McKerrow, J. D., Al-Rawi, J. M. A. & Brooks, P. Use of diphenyliodonium bromide in the synthesis of some *N*-phenyl α-amino acids. *Synth. Commun.* **40,** 1161–1179 (2010).